METHAMPHETAMINE AND AMPHETAMINE DIFFERENTIALLY AFFECT ASSOCIATIVE LEARNING: BEHAVIORAL AND NEUROBIOLOGICAL CORRELATES

BY

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DISSERTATION

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Abstract

One factor hypothesized to contribute significantly to addiction processes is maladaptive associative learning wherein substance-related cues become closely associated with the drug response. These strong associations are thought to contribute significantly to drug craving and promote the cycle of abuse by increasing susceptibility to relapse. One goal of the studies presented here was to model aspects of the addiction process by examining psychostimulant-induced alterations of associative learning, and how specific neurobiological mechanisms, namely glucocorticoid or dopamine receptor activation, contribute to these alterations. A second goal was to investigate potential differences in the effects of two psychostimulants; methamphetamine (METH) and amphetamine (AMPH). These drugs were chosen because, while structurally very similar, they have differing abuse liabilities. Some of the disparities in rates of use and abuse can be accounted for by availability, with METH being more affordable and easier to obtain than AMPH. However, increasing diversion of prescription AMPH for non-medical use is thought to contribute to the rise in prescription drug abuse indicating that it is unlikely to be just a question of greater availability of METH.

There are many methods by which the effects of psychostimulants on associative learning can be assessed, however two particular techniques, Pavlovian-to-instrumental transfer (PIT) and sensitization, were chosen for use in the studies presented here. In Experiment 1, a sensitization paradigm was utilized to determine whether repeated administration of low to moderate doses of AMPH or METH result in differing levels of locomotor sensitization, conditioned locomotor behavior, or cross-sensitization. The results of this study suggest that both drugs elicit approximately equal levels of

sensitization and conditioned behavior. However, rats that were pre-treated with METH exhibited cross-sensitization to AMPH, but rats pre-treated with AMPH did not exhibit sensitization to METH. Another important finding from Experiment 1 was that when drugs were given in the presence of salient stimuli, METH was able to modulate associative learning to a greater degree than AMPH. In Experiment 2, a PIT paradigm was utilized to determine whether AMPH and METH were capable of differentially impacting the ability of a conditioned stimulus to energize responding for an unconditioned stimulus. The results of this experiment suggest that both METH and AMPH interfered with PIT, but METH still exhibited a greater impact on associative learning than AMPH.

Differing, but sometimes overlapping, cellular and molecular events define the two phases of sensitization – expression and induction. Given that stress and subsequent glucocorticoid release can influence the sensitized response to psychostimulants, it is important to understand the mechanisms that govern these changes. In Experiment 3, I examined whether glucocorticoid receptors (GRs) contribute to induction or expression of psychostimulant-induced sensitization, as well as whether METH- compared AMPH-induced activity is differentially altered by inactivation of GRs. The results of this experiment suggest that GR activation plays relatively distinct roles in AMPH- and METH-induced sensitization.

Locomotor sensitization has been shown to be dependant upon DA transmission in the mPFC; it has not been shown whether D1 receptor activation is critical to the locomotor response to psychostimulants. In Experiment 4, I examined whether a D1 receptor blockade within the mPFC differentially alters METH- compared to AMPH-

induced locomotor activity. This was accomplished by infusing the D1 receptor antagonist SCH 23390 directly into the mPFC prior to psychostimulant administration. The results of this experiment suggest that activation of mPFC D1 receptors play a critical role in psychostimulant-induced locomotor activity, but are most likely not the mediating factor underlying differences between METH and AMPH.

In the experiments presented here, both METH and AMPH were found to be capable of altering associative learning, but METH appears to have a greater impact on these processes compared to AMPH. It is possible that these differential effects are mediated by activation of GRs. The initial locomotor response to both of these drugs is heavily dependant upon D1 receptor activation in the mPFC, but this is most likely not the mediating factor in the differences between the two drugs. These findings indicate that the differing abuse liabilities between METH and AMPH may be partially predicated by their influence on associative learning processes. Furthermore, overlapping, but somewhat distinct neurobiological mechanisms likely govern these drug-induced differences in associative learning.

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Chapter 1. Introduction

A. General Introduction

Psychostimulants, which include cocaine, amphetamine, methamphetamine, and methylphenidate, are a class of drugs that are defined by their ability to increase physical activity, heart-rate, and wakefulness, decrease appetite, and, in humans, create a heightened sense of euphoria and well-being. Use and abuse of psychostimulants is widespread across the United States. In the year prior to a 2006 SAMHSA survey, 6 million people 12 and older had admitted to using cocaine in any form (SAMHSA report 2006). Methamphetamine (METH) use and abuse is also widespread, and is considered to be at epidemic levels with epidemiological reports from DAWN (Drug Abuse Warning Network) describing high numbers of admissions for treatment in over 1/3 of states (NIDA research report 2006). In 2007 non-medical use of Adderall, a racemic mixture of *d*- and *l*-amphetamine (AMPH) often prescribed for the treatment of attention deficit and hyperactivity disorder (ADHD) (Pelham et al. 1999), was reported to be at 2.8% for all 12^{th} graders (Johnston et al. 2009).

One factor hypothesized to contribute significantly to addiction processes is maladaptive associative learning. This occurs as substance-related cues become closely associated with the drug response, with these cues eventually gaining the capacity to have a predictive value for drug-reward. Not only can drug-related paraphernalia directly related to ingestion of the substance gain predictive value, but contextual stimuli as well. These strong associations are thought to contribute significantly to drug craving and help maintain the cycle of abuse by increasing susceptibility to relapse (Field and Cox 2008). One goal of the studies presented here was to model aspects of the addiction process by

examining psychostimulant-induced alterations of associative learning, and how specific neurobiological mechanisms, namely glucocorticoid or dopamine receptor activation, may contribute to these alterations. A second goal of these studies was to investigate potential differences in the effects of two closely related psychostimulants; METH and AMPH. These two drugs were chosen because, while structurally very similar, they have differing abuse liabilities with lifetime incidence of non-medical use of METH around 5.3% with AMPH at 1.4% (Coliver et al. 2006). Some of the disparities in rates of use and abuse between the two drugs can be accounted for by availability, with illicit METH being more affordable and easier to obtain than AMPH. However, increasing diversion of prescription AMPH for non-medical use is thought to contribute to the rise in prescription drug abuse thus indicating that it is unlikely to be just a question of greater availability of METH (Pelham et al. 1999; Romanelli and Smith 2006).

The following provides an overview of the relationship between psychostimulants and associative learning processes, including some of the relevant neural circuitry, potential neurobiological mechanisms, and the primary methodology that will be utilized in the studies presented here. This is followed by an examination of studies that have directly compared METH and AMPH. Lastly the overall aims for the experiments are outlined.

B. Associative Learning and Psychostimulants

When a stimulus proceeds or coincides with a natural reward, such as food or sex, the stimulus can become associated with reward. Subsequently, these stimuli can take on predictive value for the reward, and thus the stimuli themselves gain motivational

salience to the organism. It is often postulated in theories of addiction development that addiction itself is partially due to a form of disordered learning wherein excessive motivational value is conferred onto drug-related stimuli that predict drug reward (Wikler 1973; O'Brien et al. 1992.; Di Chiara et al. 1999). This excessive motivational value – often referred to as incentive salience – conveys upon the stimuli the ability to control behavior, which is thought to lead to many of the negative activities associated with addiction such as inordinate amount of time and resources spent procuring the drug, and reduced impulse control for the use of the drug (Field and Cox 2008). These effects are hypothesized to be due to associative learning processes that are similar to Pavlovian conditioning wherein a previously neutral conditioned stimulus (CS) takes on predictive value for an unconditioned stimulus (US). After repeated pairings with the US, the CS can then take on the ability to drive behavior related to obtaining the US (Wikler 1973; O'Brien et al. 1992).

There are numerous examples within the clinical literature of associative learning between stimuli and drug-effects contributing significantly to craving and subsequent relapse in human addicts, even long after cessation of drug use (O'Brien et al. 1990). For example, in abstinent abusers exposed to pictures of drug paraphernalia, components of the limbic system and cortex, such as the amygdala and prefrontal cortex, exhibit increased glucose metabolism (Wang et al. 1999; Childress et al. 1999). Similar studies have found that exposure to these cues produce increases in neural activation, as measured by increased blood flow, in the cingulate cortex (Maas et al. 1998; Garavan et al. 2000). Furthermore, similar cues also increase dopamine in the striatum in cocaine-addicted subjects (Volkow et al. 2006; Wong et al. 2006).

Studies utilizing animal models have also provided ample evidence for the acquisition of associations between drug-taking and the cues or context that are closely linked to it. For example, in rats trained to lever press for cocaine in the presence of a light cue and then given extinction sessions where reinforcement is no longer delivered and responding subsequently ceases, re-exposure to the cocaine-associated light cue leads to reinstatement of lever-pressing behavior (de Witt and Stewart 1981). Rats are also more resistant to extinction if delivery of cocaine following lever press behavior is paired with discrete stimuli (Weiss et al. 2001). More distal cues also have the potential to drive drug-seeking behavior. For example, when cocaine and heroin (speedball) self-administration is extinguished in a context different from the one where self-administration training occurs, exposure to the original training chamber results in reinstatement of drug-seeking behavior (Crombag and Shaham 2002). In an alternate version of the reinstatement paradigm, rats that are trained to run to a goal box for a heroin infusion do so faster when the box is paired with a distinct odor. After extinction trials in the absence of the odor, adding the odor to the start box greatly increases run-time (McFarland and Ettenberg 1997).

The primary action of psychostimulants occurs in brain circuitry that has also been demonstrated to be critical for the development of associative learning (Di Chiara et al. 1999; Everitt et al. 1999; Moghaddam and Homayoun 2008). These areas are considered to be part of the reward centers, and include the mesolimbic dopamine (DA) pathway and its targets. This pathway originates with dopaminergic cell bodies in the ventral tegmental area (VTA) and has multiple interconnections with the nucleus accumbens (NAc), the amygdala, the hippocampus, and the medial prefrontal cortex

(mPFC) among others (Everitt and Robbins 2005). Collectively these areas are considered key for the executive control of actions and make up a vital processing component for goal-directed behavior (Fuster 2000; Moghaddam and Homayoun 2008). Altering DA activity within the mesolimbic circuit can increase or decrease drive for both natural and drug-related reward (reviewed in Berridge 2009). For instance, within the mPFC, decreasing DA transmission or interfering with DA receptor function alters conditional associative learning for food (Bach et al. 2008), whereas DA efflux is increased in the presence of a conditioned stimuli that had been previously paired with a food reward (Mingote et al. 2004). The relationship between psychostimulants and stimuli can be altered as well by changing DA transmission within the mPFC, with D1 receptor antagonists and 6-OHDA lesions both inhibiting psychostimulant-induced conditioned place preference (Isaac et al. 1989; Sanchez et al. 2003).

It should be noted that direct alterations in DA transmission within the mesolimbic system is not the only mechanism by which the relationship between associative learning and addiction processes can be modified. One such mechanism that has gained attention in recent years is the connection between the stress response and addiction processes. Stress, which refers to the processing of events that are possibly harmful or challenging in some way, has been demonstrated to increase response to psychostimulants and is thought to be a potential contributor to relapse of addictive behaviors (Sinha 2008; Le Moal and Koob 1997). Furthermore, individuals who report early life stress such as sexual and physical abuse are at greater risk for substance abuse (Dembo et al. 1988; Harrison et al. 1997). Alcohol consumption has been positively correlated with perceived stress levels (DeFrank et al. 1987). Interestingly, withdrawal

from drugs can create stress, and this withdrawal-induced stress is thought to contribute greatly to predisposition for relapse (Baker et al. 2004).

One of the principle adaptive responses to stress is increased release of adrenocorticotropic hormone (ACTH) from the anterior pituitary gland. Increased ACTH levels result in release of glucocorticoid hormones (Munck et al. 1984), which are cortisol in humans and corticosterone (CORT) in rodents. Glucocorticoids have a variety of functions which include acting in the immune systems as an anti-inflammatory agent (Rhen and Cidlowski 1995), contributing to glucose metabolism, as well as playing a role in organ development (Lupien et al. 2009). The effects of stress on drug response are highly correlated with changes in levels of circulating CORT. Subcutaneous administration of AMPH increases CORT levels dose-dependently, with the highest amount of CORT measured in rats exhibiting significant levels of focal stereotypy (Swerdlow et al. 1993). Administering CORT just prior to an AMPH self-administration session increased the reinforcing properties of AMPH and facilitated acquisition of AMPH self-administration (Piazza et al. 1991). Adrenalectomy to remove circulating CORT reduces locomotor activity induced by AMPH at 1.5 mg/kg, which can be reversed by subsequent CORT administration (Cador et al. 1993).

There are strong indications within the pre-clinical literature that stress can modify the drug response by altering associative learning. For example, exposure to a wide variety of stressors can result in reinstatement of previously extinguished drug-induced conditioned place preference (see reviews by Shaham et al. 2003; Weiss 2005; Aguilar et al. 2008). Furthermore, locomotor sensitization, which is considered by some to be evidence of associative learning between environmental cues and drug effects

(Robinson and Berridge 1993), appears to be susceptible to modification by stressors. Stress can pre-sensitize animals to psychostimulants, with exposure to a variety of stressors including tail pinch, footshock, and restraint prior to psychostimulant administration enhancing both the locomotor response and DA transmission within the mesolimbic DA system (Antelman 1980; Robinson et al. 1985; Sorg and Kalivas 1991; Sorg 1992). It is not clear if this effect is directly mediated by changes in CORT levels, however, as the effects of adrenalectomy on AMPH-induced sensitization have been varied – both decreases and no effect have been reported (Rivet et al. 1989; Badiani et al. 1995). The methodologies for these two studies were quite different, with Badiani et al. studying rotational behavior in 6-OHDA lesioned animals, and Rivet et al. studying intact animals in a square chamber. Size and shape of the testing chamber has been shown to alter patterns of sensitization, which may explain these inconsistencies (Ohmori et al. 2000).

C. Models of Associative Learning

There are many methods by which the effects of psychostimulants on associative learning can be assessed, however only two particular techniques, Pavlovian to instrumental transfer and sensitization, were chosen for use in the studies presented here. These techniques represent an attempt to examine several facets of the relationship between psychostimulants and associative learning that until now were relatively unexplored. The concept of sensitization, originally described as reverse tolerance, has been defined for decades. However, many of the mechanisms underlying this phenomenon are still unexplained. Thus, sensitization was utilized to examine METH- and AMPH-induced behavioral and neurobiological changes.

Based upon findings from our initial sensitization experiment, we chose to further examine the impact of METH and AMPH on associative learning. To accomplish this, a separate paradigm, known as Pavlovian-to-Instrumental transfer (PIT), was utilized to examine different aspects of associative learning. As discussed in the previous section, drug-related cues can have a profound impact on the reward-directed behavior. Even though PIT was originally described by Estes in 1943, it has only been within the past decade that is has been used as a means to further investigate how psychostimulants can modify the relationship between cues and reward-related behaviors.

The following provides an overview of the incentive sensitization and PIT paradigms, as well as some of neural circuitry and neurobiological mechanisms that are known contributing factors to these phenomena.

i. Pavlovian-to-instrumental transfer

Reward-related Pavlovian stimuli, such as drug-associated cues, can alter instrumental behavior through associative learning processes. In this case, the alteration is postulated to occur via the stimulus-reinforcer association and their influence on reward prediction (Reviewed in Everitt et al. 2001). This effect can be examined using the PIT paradigm. PIT occurs when instrumental responding is enhanced in the presence of a stimulus previously associated with a reward (Estes 1943). Typically, PIT is assessed by first training an animal on an instrumental behavior, such as lever-pressing, with food reinforcement. In the absence of levers, but in the same context, the animal is then exposed to a conditioned stimulus (CS) in combination with the receipt of a reward, such as a food pellet, which results in the conditioning of approach behavior. Lastly, the CS is presented along with the opportunity for lever pressing such that the influence of

the CS on the expression of instrumental behavior can be determined. The final stage of the paradigm is referred to as the "transfer" stage, or the PIT test.

The nucleus accumbens (NAc), a component of the ventral striatum, has been demonstrated to be important for instrumental learning, particularly in forming the relationship between action-outcome wherein an animal learns the contingency between a specific action and a reward (Reviewed in Everitt et al. 1999). It is thought that as a task is learned, the method of encoding the task moves from action-outcome to stimulus-response wherein the action becomes habit-based, defined as a stimulus presentation leading to response with a decreased emphasis on the outcome. This concept is critical to PIT since the transfer itself is dependent on two-process learning theory whereby rats respond via stimulus-response and not action-outcome (Rescorla and Solomon 1967). The transition from action-outcome to stimulus-response encoding has been demonstrated by examining the effect of outcome devaluation on rats that are early in instrumental training compared to those that are late in instrumental training. Rats that are over-trained, and are thought to be utilizing stimulus-response encoding, demonstrate greater insensitivity to outcome devaluation thus implying they respond more to the presence of the stimulus than to the possibility of reward (Adams and Dickinson 1981; also reviewed in Balleine and Dickinson 1998).

In studies utilizing the PIT technique, it has been demonstrated that lesions in the brain's reward pathway, particularly the NAc core and the central nucleus of the amygdala (CeA), produce decreases in lever press behavior during the PIT test (Corbit et al. 2001; Everitt et al. 2003; Hall et al. 2001). Blocking DA receptors within the NAc also decreases PIT (Lex and Hauber 2008), whereas intra-NAc administered AMPH

enhances PIT (Wyvell and Berridge 2000). Interestingly, when corticotrophin-releasing factor (CRF) is infused into the NAc, PIT is also increased (Pecina et al. 2006). CRF is released from the CeA, and has been demonstrated to increase locomotor activity as well as DA levels in the NAc and the medial prefrontal cortex (mPFC) (Dunn and Berridge 1987). Furthermore, a blockade of CRF-1 receptors prevents cocaine-induced DA efflux in the NAc (Lodge and Grace 2005). Taken together, these studies demonstrate the importance of the the NAc in mediating changes in associative learning strength of the motivational properties of reward.

ii. Incentive sensitization

Drug associated cues can alter not only instrumental behavior, but overall locomotor activity as well. The phenomenon termed sensitization takes place when an increase in response to the same dose of drug occurs after repeated, intermittent drug exposure and is also considered to be due, in part, to enhancements in associative learning between the environment and the psychomotor activating effects of the drugs (Robinson and Berridge 2002; Badiani and Robinson 2004). Multiple different psychomotor effects of stimulants can undergo sensitization; however locomotor sensitization is one of the more obvious effects of psychostimulant sensitization in rodents. Sensitization is often described as containing two temporally distinct phases; the induction phase, and the expression phase which follows the induction phase generally after a withdrawal period (Kalivas and Stewart 1991; Robinson and Becker 1986). The induction phase is defined as the time period during which repeated administration occurs, resulting in transient molecular and cellular changes. The changes that occur during induction over time result in enduring neural alterations from which the

augmented response known as expression arises (Pierce and Kalivas 1997). Once expression has been established, it is relatively stable with successful tests for sensitization carried out up to one year later (Mendez et al. 2009, Robinson and Berridge 1993; Paulson et al. 1991).

Locomotor sensitization has been demonstrated to be context dependent wherein the context itself becomes a CS that is predictive of drug exposure, and the extent to which an animal has been habituated to the drug-administration environment influences the magnitude of sensitization expressed (Carey and Damianopoulos 2006). If repeated administration occurs within a home-cage then sensitization is greatly reduced, however administration in a separate environment results in robust levels of sensitization (Badiani et al. 1995). Furthermore, when AMPH is administered automatically via an i.v. catheter in an animal's home-cage, thereby removing almost all drug-associated contextual cues, sensitization was completely abolished (Crombag et al. 1996; Ostrander 2003). It has even been argued that the more salient the CS, the greater the magnitude of sensitization (Badiani and Robinson 2004). Taken together, these experiments act as evidence that this effect is a function of associative learning occurring between the environment and the drug response.

Associative learning is only one aspect of locomotor sensitization, which is considered to be an expression of a larger phenomenon known as incentive sensitization. Described by Robinson and Berridge in 1993, incentive sensitization occurs when the drug associated cues (stimuli) take on motivational properties or increased salience. This increased salience then contributes to the augmented behavioral response (Robinson and Berridge 1992). While there has been some discussion as to whether locomotor

sensitization truly represents an increase in motivation for drug reward (see discussion in Robinson and Berridge 2008), there is increasing evidence for this relationship in humans (review by Leyton 2007): strong evidence for this correlation exists in pre-clinical literature. For example, rats acquire self-administration of psychostimulants faster after having previously undergone a sensitizing regiment of a psychostimulant (Piazza et al. 1990). Pre-sensitization to cocaine also shifts the dose-effect curve for development of conditioned place preference to the left (Shippenberg and Heidbreder 1995), and AMPH pre-sensitization can increase acquisition of habit-based responding (Nelson and Killcross 2006). Taken together, these experiments indicate an increased ability to associate context with reward, thus implying that motivation, or drug "wanting" as Wyvell and Berridge termed it, is increased.

Sensitization of locomotor activity is indicative of hypersensitivity, or sensitization, of dopaminergic transmission in response to psychostimulant administration within the reward pathways of the brain. Acute psychostimulant administration transiently increases DA levels within the reward pathways (Bradberry et al. 1989; Hurd et al. 1989), and after repeated intermittent administration this response becomes sensitized (Kalivas and Duffy 1990; Pettit et al 1990). The correlation between DA and locomotor activity has also been well established. When administered systemically a D1 receptor antagonist can block the expression of METH and cocaine induced sensitization (Kuribara and Uchihashi 1993; Mattingly et al. 1996). Manipulations of DA transmission, either by altering levels of DA or DA receptor activity, within parts of the mesolimbic DA pathway such as the mPFC (Bjijou et al.

2002), ventral tegmental area (Cornish and Kalivas 2001; Tanabe et al. 2004; Vezina 1996), and NAc (Todtenkopf et al. 2002), can also alter locomotor sensitization.

D. Methamphetamine and amphetamine

Not all drugs have the same liability for abuse, and differing abuse liabilities can occur for multiple reasons. However, drugs with high abuse liabilities tend to share similar features such as high reports of "liking" the drug effects, greater ease to obtain the drug, and are lower in cost (Griffiths et al. 2003). Abuse of both METH and AMPH have been the on rise for the past ten years, but currently have differing rates of abuse with lifetime incidence of non-medical use of METH is around 5.3% and AMPH at 1.4% (Coliver et al. 2006). The differing rates between the two drugs may be partially attributable to availability of drug, with illicit METH being more affordable and easier to obtain than AMPH (Romanelli and Smith 2006). AMPH, unlike METH, is most commonly obtained in prescription form, however there is increasing evidence that a large amount of prescription AMPH is being diverted for sale for non-medical use which, if the trend continues, will likely result in an increase in the number of reports of AMPH abuse (Poulin 2007).

Another possible explanation for the differing rates of abuse, which is perpetuated in both the scientific literature and the mainstream media, is that METH is a more potent psychostimulant than AMPH and this is the major factor contributing to its greater abuse potential (Feldman et al. 1997; NIDA Research Report 2006). Interestingly, a careful examination of the literature reveals that few or relatively modest differences in potency have been observed when METH and AMPH are compared within the same behavioral study. For example, rats and rhesus monkeys trained to self-administer AMPH and

METH take approximately equal amounts of the drugs (Balster and Schuster 1973; Yokel and Pickens 1973). Drug discrimination studies have shown that rats (Kuhn et al. 1974), much like humans (Lamb and Henningfield 1994), are unable to discriminate between the two drugs over a fairly wide dose range. Even when tested in an open-field chamber, animals given a single dose of 1, 2, or 4 mg/kg AMPH or METH exhibit similar increases in locomotor activity (Shoblock et al. 2003, Milesi-Halle et al. 2007).

The differences in potency that have been observed between these two drugs were primarily found at higher doses following binge-like exposure, or at the neurochemical level. For example, when 2.5 or 4 mg/kg AMPH and equimolar doses of METH were first given via 15 once-daily injections and later in "binge" treatments (once every 2 h), which results in tolerance to the locomotor activating effects of the drug, METH was shown to produce a prolonged "poststereotypy" response compared to AMPH. METH and AMPH display similar pharmacodynamic properties (Melega et al. 1995) as well as cellular activity; both drugs increase dopamine (DA) levels by acting at the DA transporter to prevent reuptake as well as by enhancing DA release. However, after a 2 mg/kg dose of METH or AMPH, AMPH was found to elevate DA, and METH elevates glutamate (GLU) within the medial prefrontal cortex (mPFC) (Shoblock et al. 2003). AMPH also released more GLU in the NAc (Shoblock et al. 2003). A binge-type exposure resulted in significantly lower levels of 5-HT in the caudate and NAc in METH-treated rats than those treated with AMPH (Segal and Kuczenski 1997). After a single 2.5 mg/kg dose of METH or AMPH, AMPH raised norepinephrine (NE) levels significantly more than METH in the mPFC (Shoblock et al. 2004).

Examinations of the effects of METH and AMPH on DA transmission in slice culture and cell culture models have resulted in mixed results similar to those from the behavioral studies. For example, in a mouse striatal slice culture AMPH was found to be a more potent DA uptake inhibitor than METH (John and Jones 2007), but in rat caudate synaptosomes METH was found to be potent at inhibiting DA uptake (Rothman et al. 2001). Additionally, in human dopamine transporter (DAT) expressing COS-7 cells, METH was more effective than AMPH at releasing DA via reversal of DAT activity (Eshleman et al. 1994). However, in a separate cell line also engineered to express human DAT, rat C6 glioma cells, no difference was found in efficacy between METH and AMPH induced DA release (Johnson et al. 1998). Lastly, in work utilizing both *in vivo* patch-clamp electrophysiology and *in vivo* voltammetry a separate group has demonstrated that METH possibly has a greater effect than AMPH on DAT activity to increase extracellular DA concentrations (Goodwin et al. 2009).

E. Aims

Aim 1. Examine the effects of METH and AMPH on associative learning.

It has been previously shown that AMPH- and METH-induced locomotor activity diverges at higher, stereotypy-inducing doses (Segal and Kuczenski 1997). However, it has not yet been established whether these drugs create differing responses after repeated exposure at low to moderate doses (0.5-1.0 mg/kg) that activate behavior but do not induce stereotypy. Given that sensitization and conditioned behavior are thought to be significant aspects of addiction-related adaptations in behavior and neurobiology, it is important to determine if repeated METH or AMPH create distinct patterns of behavioral activation. In Experiment 1, I utilized a paradigm of repeated, intermittent drug exposure

to address the following question: Does repeated administration of low to moderate doses of AMPH or METH result in differing levels of locomotor sensitization, conditioned locomotor behavior, or cross-sensitization? The results of this study, which have been published (Hall et al. 2008) and are reproduced in Chapter 2, suggest that both drugs elicit approximately equal levels of sensitization and conditioned behavior. However, rats that were pre-treated with METH exhibited cross-sensitization to AMPH, but rats pre-treated with AMPH did not exhibit sensitization to METH.

Another important finding from Experiment 1 was that when drugs were given in the presence of salient stimuli, METH was able to modulate associative learning to a greater degree than was AMPH. This suggests that the hypothesized differences in potency for the development of addiction to METH and AMPH may be the result of a differential ability of these drugs to enhance associative learning. It might be the case that these drugs differentially influence the development of CS-US associations. In Experiment 2, a PIT paradigm was utilized to address the following question: Are AMPH and METH capable of differentially impacting the ability a CS (combination light and tone) to energize responding for a US (food pellet)? Relatively few experiments have employed PIT to examine the ability of psychostimulants to modulate associative learning, so we chose this technique with the intent of examining how drug-related conditioned stimuli gain control over behavior. The results of this experiment (Chapter 3) suggest that both METH and AMPH interfered with PIT, but METH still exhibited a greater impact on associative learning than did AMPH.

Aim 2. Examine possible neurobiological mechanisms underlying psychostimulant-induced alterations in associative learning.

Differing, but sometimes overlapping, cellular and molecular events define the two phases of sensitization- expression and induction. Given that stress and subsequent glucocorticoid release can influence the sensitized response to psychostimulants (Antelman et al. 1980; Robinson et al. 1985; Sorg and Kalivas 1991; Sorg 1992), it is important to understand the mechanisms that govern these changes. When administered systemically, the GR antagonist mifepristone can attenuate AMPH- induced locomotor activity on the drug challenge (De Vries et al. 1996). However, it is unknown whether GR activation is solely involved in expression, or if it contributes to induction of sensitization as well. Furthermore, the effects of GR blockade on METH-induced locomotor activity have not been reported previously. Thus, in Experiment 3, I addressed the following questions: Do GRs contribute to induction or expression of psychostimulant-induced sensitization? Is METH-induced compared to AMPH-induced activity differentially altered by inactivation of GRs? The results of this experiment (Chapter 4) suggest that GR activation plays separate roles in AMPH- and METH-induced sensitization.

Locomotor sensitization has been shown to be dependent upon DA transmission in the mPFC; it has not been shown whether D1 receptor activation is critical to the locomotor response to psychostimulants (Ben-Shahar and Ettenberg 1998; Prasad et al. 1999; Beyer and Steketee 2002). Much work has been done to characterize the contribution of D2 receptors to induction and expression of sensitization but the role of D1 receptors remain relatively uncharacterized (Bast et al. 2002; Beyer and Steketee

2002; Steketee and Walsh 2005). In Experiment 4, which has been previously published and is reproduced here, I addressed the following question: Does blocking D1 receptors within the mPFC differentially alter METH- compared to AMPH-induced locomotor activity? This was accomplished by infusing the D1 receptor antagonist SCH 23390 directly into the mPFC prior to psychostimulant administration. The results of this experiment (Chapter 5) suggest that activation of mPFC D1 receptors play a critical role in psychostimulant induced locomotor activity, but are most likely not the mediating factor underlying differences between METH and AMPH (Hall et al. 2009).

Chapter 2.

Experiment 1 – Does repeated administration of low to moderate doses of AMPH or METH result in differing levels of locomotor sensitization, conditioned locomotor behavior, or cross-sensitization?[1]

i. Abstract

Rationale: Methamphetamine (METH) is typically characterized as a more potent psychostimulant than amphetamine (AMPH), but few studies have directly compared the effects of these drugs at low, behaviorally activating doses that tend not to produce focused stereotypy.

Objectives: To compare the effects of AMPH or METH treatment on locomotor activity in an open-field arena, focusing on their ability to produce conditioned locomotor activity, sensitization, and cross-sensitization.

Methods: Adult male rats were given AMPH or METH (0.5 or 1.0 mg/kg) for five days, with half of the rats presented with discrete, salient stimuli (S+) during the post-injection period. Following a three-day withdrawal, they were given three different injections on successive days: a saline challenge to assess conditioned responding, a drug challenge to assess sensitization, and a cross-sensitization test to the same dose of the drug with which they were not pre-treated.

Results: Except in certain conditions, AMPH and METH were equipotent at activating locomotor activity. The exceptions included when rats were presented with S+ on acute and drug challenge days, and in tests of cross-sensitization. There were no consistent

[1] This work was previously published and is reproduced here with permission from the copyright holder Elsevier. Bibliographic information: Hall DA, Stanis JJ, Marquez Avila H, Gulley JM (2008) A comparison of amphetamine- and methamphetamine-induced locomotor activity in rats: evidence for qualitative differences in behavior. Psychopharmacology 195(4): 469-78

differences in the magnitude of sensitization produced by AMPH or METH, and both drugs produced similar amounts of conditioned locomotion after a saline injection. *Conclusions*: We have found specific conditions where METH is more potent than AMPH**,** but this study and others that used higher doses of these drugs are not consistent with the generalized characterization of METH as a more potent psychostimulant.

ii. Introduction

Amphetamine (AMPH) and its N-methylated derivative methamphetamine (METH) are psychostimulants that share a nearly identical chemical structure and have similar pharmacokinetic properties (Melega et al. 1995), but have different rates of abuse. Epidemiological studies suggest the life-time incidence of non-medical METH use is 5.3%, whereas it is about 1.4% for AMPH (Colliver et al. 2006). Although this might be due in part to the greater availability of illicit METH (Romanelli and Smith 2006), it is commonly suggested that METH is relatively more potent than AMPH (Feldman et al. 1997; NIDA Research Report 2006). Empirical evidence for this assertion, however, is somewhat limited because the two drugs are typically not investigated within the context of the same study. When they are, it is often the case that AMPH and METH produce similar effects. For example, rats and rhesus monkeys trained to self-administer AMPH and METH take approximately equal amounts of the drugs (Balster and Schuster 1973; Yokel and Pickens 1973). Drug discrimination studies have shown that rats (Kuhn et al. 1974), much like humans (Lamb and Henningfield 1994), are unable to discriminate between the two drugs over a fairly wide dose range. Lastly, rats given either AMPH or

METH at 1, 3 and 4 mg/kg, exhibit similar locomotor activation in an open-field (Shoblock et al. 2003; Milesi-Halle et al. 2007).

Studies showing a more potent effect of METH relative to AMPH have focused on the differential effects of these drugs on neurochemistry and behavior following a 2 mg/kg dose given acutely, or following binge-like exposure. Specifically, a single injection of 2 mg/kg METH is more effective than 2 mg/kg AMPH at releasing glutamate in the prefrontal cortex, although AMPH more potently increases dopamine in the prefrontal cortex. Furthermore, AMPH increases glutamate concentrations in the nucleus accumbens, whereas METH does not (Shoblock et al. 2003). In this study, lower photocell activity counts were seen in the METH- compared to the AMPH-treated group, which was likely due to METH-induced increases in focused stereotypies. This dose has also been shown to differentially alter 5-HT and norepinephrine levels in the caudate and hippocampus, respectively, in rats that spent more time in focused stereotypy after METH compared to AMPH (Kuczenski et al. 1995). In a binge-exposure model, where 2.5 or 4.0 mg/kg AMPH and equimolar doses of METH were first given in 15 single daily injections and later in "binge" treatments every 2 h, METH was shown to produce a prolonged "post-stereotypy" response compared to AMPH. Associated with these behaviors was a similar effect of both drugs on dopamine levels in the dorsal striatum and nucleus accumbens, but a relatively greater effect of METH on 5-HT levels in these brain regions (Segal and Kuczenski 1997).

Given that METH has only been shown to be more potent at relatively high, stereotypy-inducing doses, the present study was undertaken to compare the effects of AMPH and METH at relatively low doses (0.5 and 1.0 mg/kg) that activate behavior but

tend not to induce stereotypy (Segal and Kuczenski 1987; Gentry et al. 2004). Another aim was to compare the magnitude of conditioned locomotor activity following a saline challenge and behavioral sensitization following a drug challenge, as well as to test for cross-sensitization between the two drugs, in groups exposed repeatedly to these drugs. In half of the groups, a discrete, salient stimulus was presented intermittently during the entire post-injection interval to assess if AMPH- or METH-induced locomotor activity was altered in a dose-specific manner relative to groups that behaved in the open-field without additional cues. For tests of cross-sensitization, rats pre-treated with AMPH were challenged with METH and those pre-treated with METH were challenged with AMPH.

iii. Materials and Methods

Animals. Male Sprague-Dawley rats (n = 68), 2.5-3.5 months old, were obtained from Harlan (Indianapolis, IN) or bred in our animal facility from Harlan stock rats. They were kept on a 12:12h light/dark cycle, with experiments performed during the light cycle. Rats were housed individually in translucent home cages for an average of 25 days before the start of experiments, during which time they were handled for 15 min on five separate occasions. They remained individually housed for the experiment's duration and were allowed free access to food and water. Experimental procedures were approved by the IACUC at the University of Illinois and were consistent with the *Principles of Laboratory Animal Care* (NIH Publication no. 85-23).

Open-Field Activity. Activity was measured in open-field arenas consisting of an acrylic box (40.6 x 40.6 x 40.6 cm) fitted with two photobeam frames (16

beams/dimension; 2.5 cm between beams; Coulbourn Instruments; Allentown, PA): the lower frame (2.5 cm above the arena floor) recorded horizontal activity (i.e., locomotion), whereas the upper frame (15 cm above the floor) recorded rearing. Each chamber was in a sound-attenuating cubicle (76 x 80 x 63 cm) that had a 76 mm speaker mounted on the inside wall and a ceiling-mounted camera between two white lights (4 W each). Located just above the lower photobeam frame was a small lamp (lens diameter = 1.3 cm) containing a 28 V LED. The speaker and LED lamp were used to deliver stimuli in certain groups (see below). Each chamber was connected to a computer running software (TruScan, Coulbourn Instruments) that recorded beam breaks (100 ms sampling rate). Digital video was captured and stored to a computer for offline analysis of stereotyped behaviors.

On the first day of the experiment, rats were moved from the colony to the testing room, where they remained in their home cage for a 30-min acclimation period. They were then placed individually in the open-field chamber for 30 min, removed and injected with saline (1 ml/kg, i.p.), and returned to the open-field for 60 min. They were subsequently returned to the colony and randomly divided into AMPH (0.5 or 1.0 mg/kg, n = 16/dose), METH (0.5 or 1.0 mg/kg, n = 16/dose), or saline (n = 4) treatment groups.

On day 2, the procedure was repeated with the following exceptions. First, rats in drug treatment groups were injected with their assigned doses of AMPH or METH. Second, half of the rats in these groups (n = 8/drug/dose) were exposed to a stimulus (S+) during the entire 60 min post-injection period. The S+ consisted of a 55 dB tone and a white LED presented in a 5-s on/5-s off pattern (360 total presentations). The remaining rats (S- group) had no additional stimuli delivered. For the next four days (days 3-6), the

procedure was repeated such that rats received five drug injections total. Following a three-day withdrawal period where they remained in the colony room, rats began three sessions of saline or drug challenges. For the first (day 10), all rats received saline injections. For the second (day 11), rats were given the same drug and dose that they received prior to withdrawal. The final challenge (day 12) was a cross-sensitization test: rats received the same dose of the alternative drug from their pre-withdrawal treatment. Thus, rats previously receiving METH were given AMPH, and those previously receiving AMPH were given METH.

A separate group (n = 4) was added to examine the effects of repeated exposure to the S+. These rats received six daily saline injections, three days without injections, and a final saline challenge. Their daily testing protocol was otherwise identical to other S+ groups.

Data Analysis. Locomotion was calculated as consecutive beam breaks in the lower frame (distance traveled, in cm); rearing was calculated as number of beam breaks in the upper frame. Activity was summed in 30- or 60-min bins. Behavior of rats given 1.0 mg/kg AMPH or METH was also scored for the existence of focused stereotypy and other qualitative aspects. Raters scored digital video of the 60 min post-injection by randomly selecting 30-s intervals every five min and assigning the following score: 1 = asleep/inactive; 2 = stationary sniffing; 3 = grooming; 4 = stationary activity, sniffing, head movement, and/or rearing; 5 = exploring; 6 = hyperactivity; 7 = hyperactivity with some repetitive movements; 8 = fast-patterned, mostly repetitive exploration; 9 = focused stereotypy, with repetitive head and/or body movements but no locomotion. Group averages of these scores were analyzed initially in successive 5-min bins. Because there

were no consistent group differences at successive time periods and the time course curves were asymptotic after the 10-min time point, these data were subsequently averaged across the 60 min post-injection period for final analysis.

The influence of saline and drug treatment on activity was first analyzed by an overall ANOVA (group x stimulus x treatment day). Follow-up analyses included mixed, two-factor ANOVA (group x treatment, with treatment as the repeated measure) for comparison of the first saline and drug treatment days and two-factor ANOVAs (dose x stimulus condition) for analysis of drug effects in the presence (S+) or absence (S-) of stimuli. When comparisons were made between drug-induced activity on the first treatment day (i.e., day 2) and saline- or drug-challenge days (i.e., days 10, 11 or 12), a mixed-factor ANOVA (treatment day x stimulus condition) was performed; one exception was for the analysis of repeated saline treatment, where one-way ANOVA was used to compare day 1 (S- group), day 2 (S+ group), and challenge (S+ group). Analysis of difference scores (i.e., behavior on challenge – acute injection days) for sensitization and conditioning effects was done with two-factor ANOVAs (dose x stimulus condition). Whenever appropriate, post-hoc comparisons of specific groups were done with Tukey tests.

Drugs. *d*-Amphetamine sulfate and *d*-methamphetamine HCl (Sigma-Aldrich; St. Louis, MO) were dissolved in sterile saline at concentrations of 0.5 or 1.0 mg/ml and injected at a volume of 1 ml/kg. Dosages were calculated as the weight of the salt.

iv. Results

AMPH and METH: Acute treatment and drug challenge following repeated exposure

The overall ANOVA (group x stimulus x treatment day) on the locomotion data indicated all main effects and two-way interactions were statistically significant (F values > 3.25, *p*-values < 0.01), whereas the three-way interaction was not. For rearing, the main effects and the stimulus x treatment day interaction were significant (F values > 3.25, *p*-values < 0.01), whereas the remaining two-way and the three-way interactions were not. Follow-up analyses revealed that acute AMPH and METH (i.e., treatment day 2) significantly increased locomotor activity and rearing compared to behavior following saline on day 1 (Fig. 2.1). Mixed-factor ANOVA revealed significant main effects and interactions for locomotion (group: $F_{7,56} = 5.43$, $p < 0.001$; treatment day: $F_{1,56} = 543$, $p < 0.001$; group x treatment day: $F_{7,56} = 4.66$, $p < 0.001$); for rearing, there was a non-significant trend for the main effect of group ($F_{7,56} = 2.36$, $p = 0.09$), a significant main effect of treatment day ($F_{1,56} = 86.3$, $p < 0.001$), and a significant interaction (group x treatment day: $F_{7,56} = 2.91$, $p < 0.05$). Analysis of individual drug effects revealed a dose-dependent effect on locomotion (Fig. 2.1A) for both drugs (main effect of dose for AMPH: $F_{1,28} = 5.35$, $p < 0.05$; for METH: $F_{1,28} = 5.20$, $p < 0.05$), with the S+ significantly augmenting drug-induced locomotor activity for rats treated with METH (stimuli: $F_{1,28} = 14.8$, $p < 0.001$) and tending to do so in those given AMPH (stimuli: $F_{1,28} = 3.93$, $p = 0.057$). The only case where METH-induced locomotion was significantly greater than that induced by AMPH was at the 0.5 mg/kg dose given in the presence of the S+ (Fig. 2.1A). Both doses of AMPH and METH increased rearing to a similar extent, but the presence of the S+ augmented METH-induced rearing (stimuli: $F_{1,28} = 8.27$, $p < 0.01$).

This was true at both doses, but was statistically significant at 1.0 mg/kg (Fig. 2.1B). METH-induced rearing was greater than that induced by AMPH in the groups given the drug with the S+, although this effect was statistically significant only at the 1.0 mg/kg dose (Fig. 2.1B).

Following repeated treatment and drug challenge, there were significant main effects of dose and stimulus for both AMPH- ($F_{1,28} = 4.35$, $p < 0.05$ and $F_{1,28} = 6.04$, $p < 0.05$, respectively) and METH-induced locomotion ($F_{1,28} = 8.51$, $p < 0.01$ and $F_{1,28} = 11.1$, $p < 0.01$, respectively), with a significant dose x stimulus interaction for METH ($F_{1,28} = 10.4$, $p < 0.01$). As shown in Fig. 2.1C, the presence of the S+ still augmented the locomotor response to METH at 0.5 mg/kg relative to the S- group; however, the same effect observed at the 1.0 mg/kg dose after acute treatment was not evident at METH challenge. At the 1.0 mg/kg AMPH dose, the enhanced locomotor activity seen in the S+ group after acute treatment was now statistically significant on AMPH challenge. In addition, METH was more potent at inducing locomotion compared to AMPH in the 0.5 mg/kg, S+ group and the 1.0 mg/kg, S- group. For rearing, the only statistically significant effect was a main effect of stimulus for rats given METH ($F_{1, 28} = 8.81$, $p < 0.01$), with the S+ enhancing METH-induced rearing at both doses. Similar to the effect observed on the acute treatment day, METH more potently induced rearing in the 1.0 mg/kg, S+ group compared to AMPH (Fig. 2.1D).

Repeated treatment led to sensitization of AMPH- and METH-induced locomotor activity, as confirmed by repeated measures ANOVA comparing distance traveled following injection on the acute and challenge days (Fig. 2.2A). Specifically, there were significant main effects of group ($F_{7, 56} = 8.39$, $p < 0.001$) and treatment day ($F_{1, 56} = 104$,

$p < 0.001$); the interaction was not significant. Post-hoc analysis indicated that nearly all groups significantly increased activity on the challenge day relative to the acute day (all p values < 0.01); the one exception was the 0.5 mg/kg METH, S- group, where the mean difference in locomotion showed a statistically non-significant trend ($p = 0.061$). Analysis of rearing data also indicated evidence of sensitization (group: $F_{7,56} = 3.64$, $p < 0.01$; treatment day: $F_{1,56} = 10.8$, $p < 0.01$), although this effect was variable: the only groups with significant increases in rearing on challenge compared to acute treatment were rats in the 0.5 mg/kg AMPH, S+ and the 1.0 mg/kg METH, S+ groups (Fig. 2.2B).

Inspection of the difference scores for mean activity on challenge and acute treatment days (Fig. 2.2) revealed apparent group differences in the magnitude of sensitization for distance traveled and rearing. The only statistically significant difference, however, was for the relatively greater effect of 1.0 mg/kg METH compared to 0.5 mg/kg METH given in the S- groups. Thus, there were no significant differences in the magnitude of sensitization produced by AMPH and METH. The ratings of behavior captured on video revealed that at the 1.0 mg/kg dose, neither AMPH nor METH produced significant stereotypy following drug challenge. For the S- and S+ groups given AMPH, the mean (± SEM) scores for the post-injection period were 6.84 ± 0.31 and 7.17 ± 0.21, respectively; for the S- and S+ given METH, they were 7.26 ± 0.24 and 7.48 ± 0.11, respectively. These values, which were not statistically significant from each other, were closest to the "hyperactivity with some repetitive movements," category.

Saline challenge following repeated AMPH or METH treatment

To determine whether conditioning occurred in response to repeated drug exposure, a saline challenge was performed on the first day following withdrawal (day 10). The presence of conditioning was defined as a statistically significant change in locomotion or

rearing during the first 30 min after injection on the challenge relative to the first saline injection (day 1). A repeated measures ANOVA revealed significant main effects of group (distance: $F_{7, 56} = 3.00$, $p < 0.01$; rearing: $F_{7, 56} = 3.01$, $p < 0.01$) and treatment day (distance: $F_{1, 56} = 99.7$, $p < 0.001$; rearing: $F_{1, 56} = 54.6$, $p < 0.001$), but non-significant interactions. Post-hoc tests revealed significant conditioning effects (all p values < 0.01) for distance traveled in all groups except the 1.0 mg/kg AMPH, S- group. For rearing, both the S- and S+ groups given the 0.5 mg/kg dose of AMPH or METH, and the S+ group given 1.0 mg/kg METH, had statistically significant effects. As shown in Fig. 2.3A and B, which depict difference scores for mean activity on saline challenge and acute saline treatment days, the only statistically significant group differences in the magnitude of conditioned behavior were between S- groups pre-treated with AMPH. Also shown in Fig. 2.3 (panels C and D) are data from a separate group of rats given repeated treatments with saline along with the S+ and challenged with saline and the S+ after a three-day withdrawal period. They show that repeated exposure to the S+ produces no statistically significant change in behavior, although there was a trend for locomotion and rearing to be reduced on the saline challenge day. Day 1 data for the rats given saline repeatedly with the S+ are not shown because an equipment malfunction resulted in the loss of data for that test session; however, the results from our complete sample of rats show that saline-induced behavior on the first day in the chamber is similar across the population.

In light of previous reports suggesting that the locomotor response to novelty (e.g., Hooks et al. 1992; Bevins and Peterson 2004) and the acute drug response (e.g., Sabeti et al. 2003) are predictive of chronic responses and conditioned locomotor effects, we

performed a correlation analysis on measures of locomotion in all of the AMPH and METH treatment groups. As shown in Figure 2.5, there were significant positive correlations between locomotion during the first 30 min that rats were in the open-field on day 1 (i.e., inescapable novelty response) and responses to the 1.0 mg/kg dose of AMPH on the acute and challenge days. This relationship was not present at 0.5 mg/kg AMPH or at either dose in METH-treated rats. Furthermore, novelty was generally not related to conditioned locomotion or to the magnitude of sensitization for either drug. Activity during the 60-min period following drug injection on day 2 (i.e., acute response) was not significantly correlated with conditioned locomotion for any of the groups, and the only statistically significant correlation with activity on drug challenge was for 1.0 mg/kg AMPH and 0.5 mg/kg METH. The most consistent relationship was between the acute drug response and sensitization. This was clearer when individual differences in initial sensitivity to drug-induced locomotion were controlled for by using a normalized sensitization score (the difference score for the challenge and acute treatment days divided by post-injection activity on the acute day). There were statistically significant, negative correlations for all groups except those getting 0.5 mg/kg AMPH with the S+. That is, as the initial response to AMPH and METH increased, the amount of sensitization produced after repeated treatment decreased. With only a few exceptions (e.g., the relationship between novelty and conditioning for the 0.5 mg/kg AMPH group), the addition of the S+ had no systematic effect on the observed correlations.

Cross-sensitization

The final challenge test was to determine the extent of cross-sensitization in the different treatment groups. Rats that were pre-treated with 0.5 or 1.0 mg/kg AMPH were given the same dose of METH, whereas those pre-treated with 0.5 or 1.0 mg/kg METH

were given the same dose of AMPH. The presence of cross-sensitization was defined as a statistically significant increase in activity during the cross-exposure challenge relative to the activity measured in the group receiving the same drug and dose on their first drug exposure day. Compared to rats given an acute injection of AMPH, those pre-treated with the same doses of METH and challenged with AMPH exhibited enhanced locomotion, or cross-sensitization (Fig. 2.4A). ANOVA revealed a significant main effect of treatment group (0.5 mg/kg: $F_{1, 28} = 14.7$, $p < 0.001$; 1.0 mg/kg: $F_{1, 28} = 24.5$, $p < 0.001$), a significant main effect of stimuli ($F_{1, 28} = 15.3$, $p < 0.001$) for the 0.5, but not the 1.0 mg/kg dose, and non-significant interactions for both doses. Post-hoc tests indicated the magnitude of cross-sensitization at 0.5 mg/kg was the greatest in rats pre-treated with METH and challenged with AMPH in the presence of the S+. At 1.0 mg/kg, both S- and S+ groups exhibited significant cross-sensitization, and this effect was to equivalent magnitudes (Fig. 2.4A). For rearing, only the main effect of treatment group at 1.0 mg/kg was significant ($F_{1, 28} = 5.63$, $p < 0.05$). As shown in Fig. 2.4B, the only statistically significant cross-sensitization of rearing was in the 1.0 mg/kg group treated and challenged in the presence of the S+.

Rats pre-treated with AMPH and challenged with METH showed less evidence of cross-sensitization (Fig. 2.4C and D). For locomotion at 0.5 mg/kg, there were significant main effects of treatment group ($F_{1,28} = 4.44$, $p < 0.05$) and stimulus ($F_{1,28} = 7.77$, $p < 0.01$), and a significant interaction ($F_{1,28} = 5.86$, $p < 0.05$); for rearing at 0.5 mg/kg, there was a significant main effect of treatment group ($F_{1,28} = 14.8$, $p < 0.05$), a significant interaction ($F_{1,28} = 8.47$, $p < 0.01$), but a non-significant main effect of stimulus. Post-hoc analysis revealed these significant effects were due largely to the

locomotion cross-sensitization in the S- group (Fig. 2.4C) and rearing cross-sensitization in the S+ group (Fig. 2.4D). For locomotion and rearing at 1.0 mg/kg, there were no statistically significant effects.

v. Discussion

Comparing AMPH- and METH-induced locomotor activation

After acute administration, the S+ enhanced drug-induced locomotion in both AMPH- and METH-treated rats, and enhanced rearing in those given METH. Between-drug comparisons revealed METH was more potent than AMPH at the 0.5 mg/kg dose for locomotion and the 1.0 mg/kg dose for rearing in the presence of the S+. The drugs were approximately equipotent in S- groups. Following repeated treatment and drug challenge, the S+ continued to enhance activity relative to S- groups. Furthermore, METH was still more potent than AMPH at the 0.5 and 1.0 mg/kg doses for locomotion and rearing, respectively. Unlike the acute treatment day, however, the 1.0 mg/kg dose of METH was more potent at inducing locomotion compared to AMPH in S- groups. Taken together, these results suggest that relatively low doses of METH are more potent than equivalent doses of AMPH at inducing locomotion and rearing in the open-field, but this effect is subject to the influence of contextual cues. This influence on METH-induced behavior is perhaps relevant for understanding human patterns of METH use and abuse. For example, it is intriguing to consider that a contributing factor to the worldwide rise in problem METH use (Rawson and Condon 2007) might be the documented shift to a route of administration (i.e., injection and smoking) that offers more opportunity for associations between drug effects and salient cues compared to the oral route of administration that is common for AMPH.

Sensitization after repeated treatment and withdrawal

Locomotor sensitization was observed for AMPH and METH at both doses and in both S+ and S- groups, with no consistent between-group differences. The presence of the light/tone stimulus did contribute, albeit in a non-systematic way, to rearing sensitization: the effect was only observed in S+ groups treated with 0.5 mg/kg AMPH or 1.0 mg/kg METH. Between-drug comparisons of locomotion and rearing sensitization revealed that AMPH and METH were approximately equipotent. Sensitization at these doses has been described before (e.g., Tilson and Rech 1973; Bevins and Peterson 2004), but a direct comparison between AMPH and METH has not. This is an important contribution of the present study because locomotor sensitization is influenced significantly by multiple procedural variables (Archer 1973; Walsh and Cummins 1976) and comparisons between studies can be difficult to interpret.

The finding that sensitization was not systematically influenced by the presence of the S+ was not surprising in light of previous studies showing that the salience of the environmental context where the drugs are administered is particularly robust (Badiani and Robinson 2004) and is difficult to influence by the addition of secondary cues (Panlilio and Schindler 1997; Crombag et al. 2000). It is not clear, however, why the S+ enhanced responses to both AMPH and METH. It might be the case that the addition of the S+ after the first drug treatment adds novelty to the environmental context, which results in a reduction in rats' relative amount of habituation. This is important because the extent to which an animal has been previously habituated to a testing environment influences the magnitude of drug-induced behavior that is expressed (Carey and Damianopoulos 2006). By enhancing behavior on the first drug day, the baseline from

which sensitization developed may have shifted, such that the overall magnitude of sensitization remains similar in S- and S+ groups.

There was a statistically significant, negative correlation between the acute drug response and sensitization. In other words, as the initial response to AMPH and METH increased, the amount of observed sensitization decreased. This relationship was strongest and most consistent across groups when the magnitude of sensitization was normalized to control for individual differences in the acute response. Initial response to AMPH has been shown to positively correlate with subsequent AMPH responses in previous studies (e.g., Bevins et al. 1997), and a similar relationship was shown recently for METH (Bevins and Peterson 2004). However, our results are similar to those reported previously for cocaine (Sabeti et al. 2003).

Conditioned activity following saline challenge

We observed conditioned locomotion in most groups pre-treated with 0.5 or 1.0 mg/kg AMPH or METH. Conditioned rearing was limited mostly to groups receiving the lower doses of AMPH or METH; repeated 1 mg/kg METH elicited conditioned rearing in the S+ group only. The only significant difference in the magnitude of conditioned activity was seen in AMPH-treated rats in S- groups: those given 0.5 mg/kg showed more activity on the saline challenge day than those given 1.0 mg/kg. Previous studies have also described conditioned locomotor activity in rats pre-treated with these doses of AMPH or METH (Tilson and Rech 1973; Bevins and Peterson 2004), but the present results demonstrate that the conditioned effects produced by the two drugs are approximately equal. Pairing repeated drug injections with the S+ had no consistent effects on conditioned responding. It was previously shown that repeatedly pairing an S+ with cocaine injections led to conditioned responding to the S+ alone (Panlilio and

Schindler 1997; Hotsenpiller et al. 2001), but these studies used procedures that specifically enhanced conditioning to the S+ and prevented generalization to the environmental context. As noted above, the salience of the environmental context of the open-field arena in the present study was likely sufficient for the development of a conditioned association with the effects of AMPH and METH.

Cross-Sensitization

Cross-sensitization between AMPH and METH, which to our knowledge has not been described in the literature previously, occurred most robustly in groups given repeated treatments of METH and then challenged with AMPH. This was the case primarily for locomotion, which was evident at both doses and was largely uninfluenced by the S+. In rats given repeated AMPH and challenged with METH, however, evidence for cross-sensitization was only observed at the 0.5 mg/kg dose. Given that METH is rapidly metabolized to AMPH in the brain (e.g., Melega et al. 1995), it was not surprising that repeated METH treatment would produce cross-sensitization to AMPH. It was somewhat unexpected, however, to find that repeated AMPH failed to produce similar cross-sensitization to METH, especially because multiple previous studies, and several of the results of the current study, suggest these drugs have similar behavioral and pharmacological effects. A contributing factor for METH's relative greater potency here might be related to its differential effects on neurochemistry in comparison to AMPH. This includes METH's greater ability to induce 5-HT (Segal and Kuczenski 1997) and glutamate release (Shoblock et al. 2003) in key brain areas. It is also notable that methylphenidate, which is a psychostimulant like AMPH but has different

pharmacodynamic properties, also does not cross-sensitize to METH (Kuczenski and Segal 2002).

Novelty

For both AMPH and METH, the locomotor response to inescapable novelty has been shown to be predictive of chronic drug responses and conditioned locomotor effects (e.g., Hooks et al. 1992; Bevins and Peterson 2004). Here, we found that novelty correlated significantly with acute and drug challenge in groups given 1.0 mg/kg AMPH, but not 0.5 mg/kg AMPH or either dose of METH. Novelty was not systematically correlated with measures of sensitization, however. The significant relationship at 1.0 mg/kg AMPH is consistent with previous studies (Hooks et al. 1991; Bevins et al. 1997), but the lack of correlation for the other dose of AMPH, both doses of METH, and sensitization in most groups, contrasts with others (Hooks et al. 1992; Bevins and Peterson, 2004). The source of these discrepancies between the present and previous studies is not clear, although it is likely not due to an influence of the S+ on the observed correlations. One contributing factor might be differences in the size of the open-field test apparatus, which can significantly influence the expression of drug-induced behavior (Walsh and Cummins 1976; Rebec and Bashore 1984). Regardless of its source, this inconsistency suggests that inescapable novelty is a predictor of AMPH- and METH-induced behavior only under particular circumstances.

Summary

We found that relatively low doses of AMPH and METH are equipotent at inducing locomotor activation after acute or challenge injection, and conditioned locomotion to saline after repeated treatment. In the presence of discrete, salient stimuli, however, the

response to acute or challenge injections of METH, and to a lesser extent AMPH, was enhanced, and the overall magnitude of activity was higher in METH- compared to AMPH-treated rats. Furthermore, repeated treatment with METH produced robust cross-sensitization to AMPH, but repeated AMPH treatment resulted in minimal cross-sensitization to METH. These results, in addition to studies with higher doses of these drugs (Shoblock et al. 2003; Segal and Kuczenski 1997), suggest that there are certain conditions where METH is more potent than AMPH at stimulating behavior, but the common characterization of METH as a more potent psychostimulant is not consistent with the available experimental evidence.

vi. Figures and Figure legends

Figure 2.1

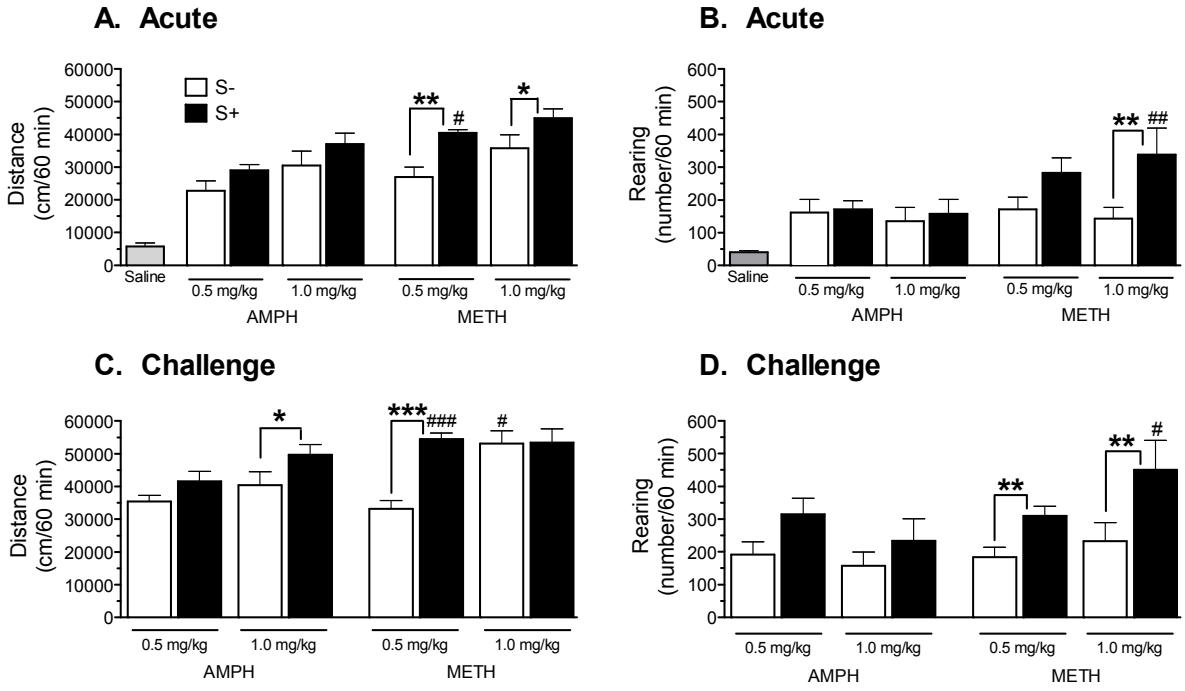

Figure 2.1 Cumulative (mean ± SEM) saline- or drug-induced locomotion (**A**, **C**) and rearing (**B**, **D**) for the 60-min period following injection (n = 8 rats/group). Shown in **A** and **B** are the data obtained on treatment day 1, when all rats received saline, and day 2, when they received AMPH or METH (0.5 or 1.0 mg/kg) in the presence (S+) or absence (S-) of a compound stimulus (flashing light and tone). ANOVA revealed no significant difference in the response to saline between groups, so these data were collapsed for presentation. Both doses of AMPH and METH significantly increased behavior over saline. * $p < 0.05$; ** $p < 0.01$; $^{\#}$ $p < 0.05$, compared to 0.5 mg/kg AMPH, S+ group; $^{\#\#}$ $p < 0.01$, compared to 1.0 mg/kg AMPH, S+ group. Shown in **C** and **D** are data obtained after AMPH or METH challenge (treatment day 11). For distance: * $p < 0.05$; *** $p < 0.001$; $^{\#}$ $p < 0.05$, compared to 1.0 mg/kg AMPH, S- group; $^{\#\#\#}$ $p < 0.001$, compared to 0.5 mg/kg AMPH, S+ group. For rearing: ** $p < 0.01$; $^{\#}$ $p < 0.05$, compared to 1.0 mg/kg AMPH, S+ group.

Figure 2.2

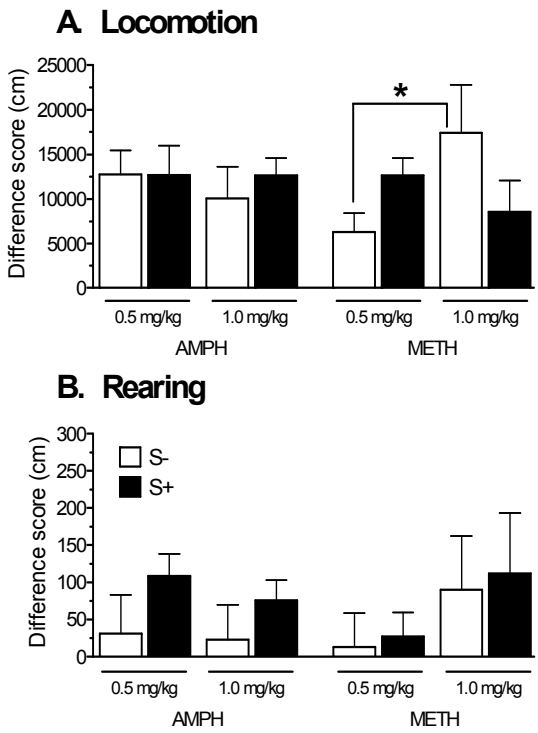

Figure 2.2 Sensitization to AMPH- and METH-induced locomotion (**A**) and rearing (**B**), represented as the difference between activity during the 60-min period following the challenge (day 11) and acute (day 2) injections (n = 8 rats/group). Statistical analysis, which was performed on the raw data from the two injection days (see Results), revealed statistically significant sensitization for locomotion in all groups except the 0.5 mg/kg METH, S- group; sensitization to drug-induced rearing was only evident in the 0.5 mg/kg AMPH, S+ and the 1.0 mg/kg METH, S+ groups. The only significant difference in the magnitude of sensitization was between the 0.5 and 1.0 mg/kg dose of METH in the absence of the stimuli (S-). * $p < 0.05$

Figure 2.3

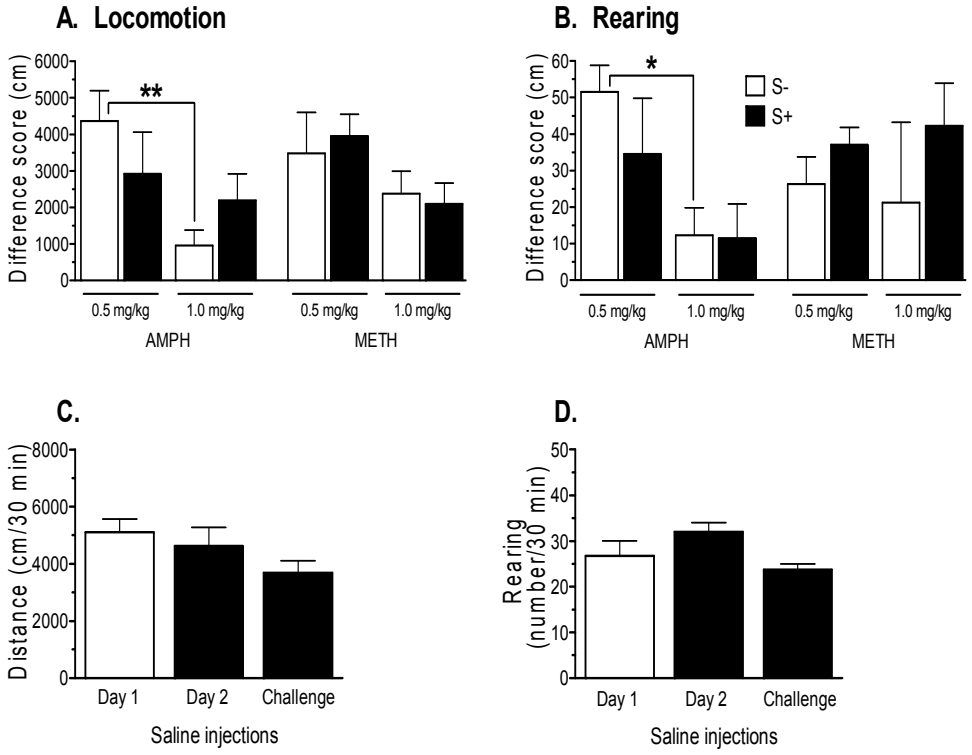

Figure 2.3 Locomotion and rearing following saline injection in rats treated repeatedly with AMPH or METH (**A**, **B**) or saline (**C**, **D**). **A**, **B**: Conditioned activity in the AMPH- or METH-exposed groups is represented as the difference between behavior following challenge (day 10) and acute (day 1) injections of saline, for the first 30 min following injection. Statistical analysis, which was performed on the raw data from the two injection days (see Results), revealed statistically significant conditioned locomotion in all groups except the 1.0 mg/kg AMPH, S- group; conditioned rearing was observed at the 0.5 mg/kg dose of both AMPH and METH, but only in the S+ group given METH at the 1.0 mg/kg dose. Due to an equipment malfunction, the 1.0 mg/kg AMPH, S- group is n = 7, while all other groups are n = 8. * $p < 0.05$; ** $p < 0.01$. **C**, **D**: Shown are data obtained from rats administered saline on Day 1 (n = 64) and a separate group (n = 4) given saline in the presence of stimuli (S+) on the second treatment day and again on a challenge day following repeated saline treatment.

Figure 2.4

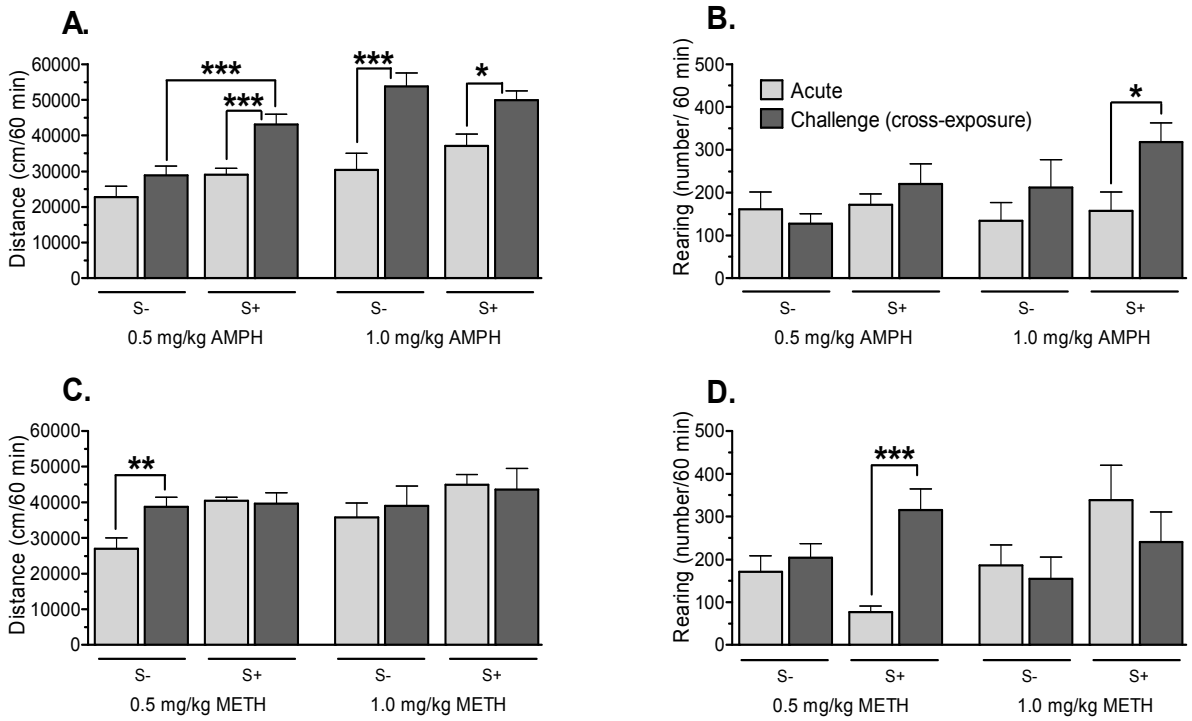

Figure 2.4 Locomotion and rearing in rats (n = 8/group) treated repeatedly with METH and challenged with AMPH (**A**, **B**) or treated repeatedly with AMPH and challenged with METH (**C**, **D**). Shown is the cumulative behavior for the 60-min following injection for "Acute" groups (i.e., those given the noted drug/dose for the first time) and "Challenge" groups (i.e., those given a challenge with the same dose, but different drug from that which they were treated repeatedly). Cross-sensitization was defined as a significant increase in behavior in the Challenge compared to the Acute group. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.01$.

Figure 2.5 Pearson correlations (*r*) between locomotion during inescapable novelty or following the first drug injection and measures of locomotion after injection (saline or drug).

	0.5 mg/kg AMPH		1.0 mg/kg AMPH		0.5 mg/kg METH		1.0 mg/kg METH	
	S-	S+	S-	S+	S-	S+	S-	S+
Novelty								
Conditioning	-0.275	-0.928**	0.363	0.323	0.192	-0.141	-0.112	0.026
Acute drug	0.542	-0.316	0.677	0.766*	-0.492	-0.077	-0.372	0.641
Drug challenge	-0.024	-0.489	0.717*	0.805*	-0.282	-0.339	0.415	0.551
Sensitization (diff.)	-0.613	-0.268	-0.052	0.016	0.371	-0.232	0.576	0.144
Sensitization (norm.)	-0.735*	-0.216	-0.319	-0.434	0.269	-0.227	0.276	0.003
Acute Drug								
Conditioning	0.496	0.447	-0.047	0.293	0.281	0.481	0.227	-0.095
Drug challenge	0.447	0.125	0.683	0.823*	0.730*	-0.421	0.071	0.554
Sensitization (diff.)	-0.804*	-0.438	-0.518	-0.354	-0.564	-0.714*	-0.704*	-0.139
Sensitization (norm.)	-0.918*	-0.589	-0.753*	-0.831*	-0.774*	-0.778*	-0.919*	-0.405

Cumulative locomotion during the 30 min following the first exposure to the open-field arena (Day 1 of testing) was used to define each rat's novelty response. Conditioning was defined as the difference between locomotion during the 30-min period after saline injections on the acute and challenge days (Days 1 and 10, respectively). Cumulative locomotion during the 60-min period after AMPH or METH injection was used for measures of drug-induced behavior (acute and drug challenge). For sensitization, two measures were used: the difference (diff.) in locomotion for the 60-min period after injection on challenge and acute treatment days, and a normalized (norm.) measure that controlled for individual differences in the acute response to AMPH or METH by dividing the difference score by the post-injection activity measured after the first injection.

Chapter 3.

Experiment 2 – Does administration of psychostimulants during the Pavlovian approach phase of a Pavlovian-to-instrumental transfer paradigm alter the transfer effect?

i. Abstract

A significant contributing factor to drug addiction is the associative learning that occurs between the unconditioned stimuli that are part of the drug-taking experience (e.g., interoceptive effects), the various conditioned stimuli (CS) that come to be associated with the unconditioned stimuli (US) (e.g., a smoking pipe), and the responses that result (e.g., drug seeking and taking). There are indications that methamphetamine (METH) alters associative learning to a greater degree than AMPH, despite having very similar pharmacokinetic and pharmacodynamic properties. The Pavlovian-to-instrumental transfer (PIT) paradigm provides a means to study these associative learning processes and to determine how particular drugs may modulate the relationship between stimuli and reward-related behavior. PIT occurs when instrumental responding is enhanced in the presence of a CS that was paired previously with delivery of reward. In the current study, this task was utilized to test the hypothesis that post-Pavlovian approach training exposure to METH or AMPH has the potential to modulate acquisition of Pavlovian approach and/or the transfer effect. Adult, male Sprague-Dawley rats first underwent instrumental training over 10 days to lever press for a food pellet. During the following six days, they underwent daily Pavlovian approach training, which consisted of 30 s pairings of a CS (light and tone) and food pellet delivery. On days 1, 3, and 5 of this training, immediately following each session, rats received either saline or drug (0.5, 1.0, or 3.0 mg/kg, i.p.). On the PIT test day, the influence of the Pavlovian CS on instrumental

behavior was examined under extinction conditions. There was no evidence of drug-related enhancement of Pavlovian approach compared to the saline treated group either during the Pavlovian approach sessions or on the PIT testing day. However, analysis of lever-pressing during the PIT test revealed disruption of PIT by both drugs, but to a greater extent by METH than AMPH. These results indicate that psychostimulant administration immediately following Pavlovian approach training sessions disrupts, rather than enhances PIT.

ii. Introduction

A significant contributing factor to drug addiction is the associative learning that occurs between the unconditioned stimuli (US) that are part of the drug-taking experience (e.g., interoceptive effects), the various conditioned stimuli (CS) that come to be associated with the US (e.g., a smoking pipe), and the responses that result (e.g., drug seeking and taking). This associative learning is thought not only to contribute to the development of addiction, but also to relapse due to enhanced retrieval of memory for drug responses that is triggered by drug-related cues (Field and Cox 2008). Strong evidence for this drug-induced enhancement exists in the preclinical literature. For example, it has been established that drug-related CSs can take on the ability to enhance both drug-seeking instrumental behavior and reinstatement in paradigms modeling relapse (Taylor et al. 2009).

The Pavlovian-to-instrumental transfer (PIT) paradigm provides a means to study how associative learning processes can subsequently affect behavioral output. PIT is said to occur when instrumental responding (e.g., lever pressing) is enhanced in the presence

of a CS that was paired previously with delivery of reward, thus resulting in a transfer of excitatory impact between the stimulus and instrumental behavior (Estes 1948; Lovibond 1983; Balleine and Dickinson 1998). The procedure for PIT occurs in three distinct phases: one is instrumental training wherein the association between a specific action and the receipt of a reward is established, a second is Pavlovian conditioning wherein a CS is presented contingently with receipt of the US, and lastly is the PIT test where the ability of the CS to increase instrumental responding is evaluated. The presence of the transfer effect is thought to be indicative of the ability of the CS to take on incentive salience, thus providing motivation for enhanced instrumental responding (Dickinson et al. 2000).

The presence of PIT is highly dependent upon activity within the nucleus accumbens (NAc) with both lesions of the NAc and local blockade of DA receptors reducing PIT (de Borchgrave et al. 2002; Lex and Hauber 2008). Conversely, infusion of an indirect DA agonist directly into the NAc enhances PIT (Wyvell and Berridge 2000). A similar result is obtained following intra-NAc infusion of corticotropin-releasing factor (Pecina et al. 2006). The influence of the NAc on PIT is highly relevant since altered activity of the NAc has been heavily implicated in addiction processes (Robbins et al. 2008), and there is increasing evidence that psychostimulants are able to profoundly impact the PIT response. For instance, when rats are pre-sensitized to AMPH but tested in a drug-free state, the transfer effect is enhanced (Wyvell and Berridge 2001).

Previous reports indicate that administration of AMPH immediately following Pavlovian approach sessions can enhance acquisition of the CS-US relationship, possibly by increasing memory consolidation (Simon and Setlow 2006; Blaiss and Janak 2007). Since increases in associative learning are implicated in addiction processes, it is

important to understand whether the increased association between CS and US is able to modify reward-related behavior at a later time-point. In the current study, we addressed this issue by utilizing the PIT task to determine if exposure to methamphetamine (METH) or AMPH following Pavlovian approach training sessions would alter the incentive value of the CS and thus modulate the instrumental response during the PIT. We chose to examine METH and AMPH because previous work from our lab has suggested that they have differential ability to modulate associative learning in the presence of salient stimuli (Hall et al. 2008). Furthermore, these drugs have different abuse liability (Colliver et al. 2006) even though their chemical structures differ by only a single methyl group and they have very similar pharmacokinetic and pharmacodynamic properties (Melega et al. 1995).

iii. Materials and Methods

Animals. Male Sprague-Dawley rats, 2.5-4 months of age, were obtained from Harlan (Indianapolis, IN, USA) or bred in our animal facility from Harlan stock rats. They were kept on a 12:12-h light/dark cycle, with experiments performed during the light cycle. Rats were housed individually in translucent home cages either upon arrival from Harlan or starting at ~2 months of age. Before experiments began, rats were handled individually for 15 min on five separate occasions. Water was available *ad libitum* throughout the study. Food was available *ad libitum* until 5 days prior to the start of the experiment, at which time food was restricted so that weight was reduced to ~85% of free-feeding weight. All rats were maintained at 85-90% of their free-feeding weight throughout the duration of the study. Experimental procedures were approved by the

Institutional Animal Care and Use Committee at the University of Illinois and were consistent with the Principles of Laboratory Animal Care (NIH Publication no. 85-23).

Apparatus. Operant behavior was monitored using standard conditioning chambers (Coulbourn Instruments) located within sound attenuating cubicles equipped with ventilation fans. One wall of the chamber contained two retractable levers that were positioned on either side of a centrally located food trough. White cue lights were located above each lever and a tone speaker (2.9 kHz Sonalert) was located directly above the food trough. Entries into the food trough were monitored by infrared detectors. A white house-light (4 W), which was illuminated during all training and test sessions, was located near the top of the chamber on the opposite wall from the food trough.

Instrumental training. The PIT paradigm utilized here was adapted from previous studies (Wyvell and Berridge 2000; El-Amamy and Holland 2007; Lee and Everitt 2008). All rats received a single 30 min session of magazine training to shape them to eat from the food trough and habituate them to the chambers. Food pellets (45 mg, Bioserv; Frenchtown, NJ, USA) were delivered on a random time (RT) 30 sec schedule. Over the next ten days, rats underwent instrumental training for 60 min/day. Both levers were extended into the chamber during these sessions, but only one was active. Assignment of the active lever to the left or right side of the food trough was counterbalanced across rats. Responses on the active lever were reinforced with food pellet delivery using the following schedule: continuous reinforcement (sessions 1-2), random ratio (RR) 2 (session 3), continuous reinforcement (session 4), RR2 (sessions 5-6), RR5 (sessions 7-8), and RR10 (sessions 9-10). During these sessions, responses on the inactive lever were recorded but had no programmed consequences.

Pavlovian approach. Following instrumental training, all animals received six daily sessions of Pavlovian approach training in the same operant chambers but with the levers retracted. Each session consisted of 10, 30 sec presentations of the CS (2.9 kHz tone and illumination of both cue lights), with pellet delivery occurring on an RT 20 sec schedule during the final 20 sec of the presentation. The CS presentation was followed by an interstimulus interval of 2-4 min. Immediately following sessions 1, 3, and 5, all rats received an injection (i.p.) of either 0.9 % saline (1 ml/kg), AMPH (0.5, 1.0, or 3.0 mg/kg), or METH (0.5, 1.0, or 3.0 mg/kg). This schedule and timing of drug administration was chosen based upon previous work showing that AMPH given in this manner, but not 2-6 hours after Pavlovian approach training, results in increased conditioned responding (Simon and Setlow 2006; Blaiss and Janak 2007).

PIT test. The day after the last session of Pavlovian training, rats received one 30 min instrumental training "reminder" session (RR 10). On the following day, they were placed in the test chambers with both levers extended. The CS was then presented three separate times with each presentation followed by a 2-4 min interstimulus interval. The session was done under extinction conditions (i.e., no food pellet delivery).

Data Analysis. Dependent measures included rate of lever pressing (number/min), ratio of trough entries during Pavlovian approach, and ratio of trough entries and lever presses during the PIT test. The ratios were calculated by determining the difference between trough entries or lever presses during the 30 s CS and the 30 s prior to the CS and dividing this by the total number of trough entries or lever presses during both intervals. Ratios from the Pavlovian approach phase were compared using either repeated measure ANOVAs (day) or mixed factor ANOVA (treatment x session,

with session as the repeated measure). Number of lever presses per minute was calculated for instrumental training and reminder sessions and was examined using single-factor ANOVAs (treatment), as were ratios from the PIT test. Lever presses and trough entries during the PIT test were examined using mixed factor ANOVA (treatment x time, with the 30 s prior to CS onset and 30 s of the CS presentation as the two levels of the repeated measure). Whenever appropriate, post-hoc comparisons of specific groups were done with Bonferonni tests.

Drugs. *d*-Amphetamine sulfate and *d*-methamphetamine HCl (Sigma-Aldrich; St. Louis, MO) were dissolved in sterile saline at concentrations of 0.5, 1.0, or 3.0 mg/ml and injected at a volume of 1 ml/kg. Dosages were calculated as the weight of the base.

iv. Results

Following ten days of instrumental training, rats in all groups exhibited high levels of lever pressing behavior (Fig. 3.1A). Comparison of the mean number of responses on the final session revealed no significant differences between groups ($F_{6, 69}$ = 0.06, p = 0.999, Fig 3.1A). Additionally, analysis of the instrumental reminder session revealed that pre-treatment with METH or AMPH during the Pavlovian approach phase did not alter motivation for reward as measured by rate of instrumental responding during the lever press reminder session ($F_{6, 63}$ = 0.197, p = 0.977; Fig 3.1B).

Figure 3.2 shows the Pavlovian approach ratio plotted across days. To determine whether conditioning occurred for each group, the ratio across days was analyzed via a single-factor repeated measure ANOVA. All groups exhibited acquisition of Pavlovian conditioned approach behavior as determined by a significant main effect of session ($F_{5,}$

$_{59}$ > 2.99, p <0.05) except METH at 1.0 mg/kg which only exhibited a trend towards a main effect of day (p = 0.069). Although upon first inspection it appears that the approach ratio was enhanced by drug, analysis revealed only a main effect of session ($F_{6, 63} = 29.9$, $p < 0.001$), but no treatment or session x treatment interaction effects.

As shown in Fig. 3.3, PIT was apparent only in groups given saline following Pavlovian approach training sessions and not in any of the drug treated groups (Fig. 3.3A). For these analyses, expression of PIT was determined by analysis of lever pressing rate for the 30 sec period prior to CS onset compared to the 30 sec period during CS presentation. ANOVA revealed a significant main effect of time ($F_{1, 141} = 19.5$, $p <$ 0.001) as well as a significant time x treatment interaction ($F_{6, 141} = 4.00$, $p < 0.01$). In contrast, treatment with 1.0 and 3.0 mg/kg METH or 3.0 mg/kg AMPH during Pavlovian approach training resulted in significant decreases in lever pressing during CS presentations on the PIT test. Subsequent examination of the PIT ratio revealed a significant effect of treatment ($F_{6, 69} = 2.43$, $p < 0.05$), with the greatest inhibition of PIT observed in the group treated with the highest dose of METH. These effects did not appear to be related to significant changes trough entries during the PIT test, as most groups increased trough entries during the presentation of the CS (Fig. 3.4A). ANOVA on these data revealed a significant main effect of time ($F_{1, 141} = 22.6$, $p < 0.001$), but no main effect of treatment, but a treatment x time interaction effect ($F_{6, 141} = 3.25$, $p < 0.01$). This interaction effect is most likely due to the METH 1.0 group which did not exhibit a significant increase in trough entries across time. Comparison of the approach ratio revealed no significant main effects.

v. Discussion

The PIT paradigm examines whether a CS that has been previously conditioned with association for a reward takes on the ability to energize instrumental responding (Estes 1948; Lovibond 1983; Balleine and Dickinson 1998). The presence of transfer is thought to be indicative of a motivational state in which a representation of the reward by the CS drives the execution of an instrumental action. The results presented here suggest that administration of METH or AMPH immediately following Pavlovian approach training sessions interferes with the transfer effect in a general PIT paradigm. Specifically, presentation of the CS either had no effect on, or significantly decreased the rate of instrumental responding in drug-treated groups. The greatest decreases were observed in groups that received the highest doses of either METH or AMPH.

We chose to administer drugs immediately following Pavlovian approach sessions due to evidence that this method results in increased CS-US associations (Simon and Setlow 2006; Blaiss and Janak 2007). While all groups demonstrated acquisition of conditioning, we did not observe any increases in our drug-treated groups compared to saline-treated controls. We hypothesize that the lack of significant effect of post-training drug administration observed here was likely a result of specific methodological differences, namely differences in length of CS presentation. Our choice of a 30 sec CS presentation was based upon work utilizing an identical CS length for PIT (Wyvell and Berridge 2000; Wyvell and Berridge 2001), however timing of the CS can have a profound impact on Pavlovian conditioning and PIT. It has been demonstrated that shorter CS presentations, such as those utilized by Simon and Setlow to elicit Pavlovian approach, are optimal for acquisition of Pavlovian approach, but longer CS presentations

greater than 30 s are more effective at eliciting PIT (Crombag et al. 2008; Delameter and Holland 2008). Therefore we suggest that the length of the CS presentation utilized here likely reduced the sensitivity of the paradigm to reveal differences in Pavlovian approach.

It was initially expected that exposure to psychostimulants following Pavlovian approach sessions would enhance approach acquisition, thus enhance the acquisition of incentive salience towards the CS and increase the likelihood of an increased transfer effect. This hypothesis was based upon both the evidence that post-session AMPH increases conditioning (Simon and Setlow 2006; Blaiss and Janak 2007), as well several indications that exposure to AMPH, either in the form of a sensitizing regimen or intra-NAc infusion prior to the PIT test, enhances PIT (Pecina et al. 2006; Lex and Hauber 2008). However, the opposite effect was observed as we found a decrease in instrumental behavior during CS presentations. Statistically, this effect was somewhat independent of dose. However, the largest decreases in PIT ratio were observed in groups that received the highest dose of METH. Previous work from our lab has indicated that METH may have a greater influence on associative learning processes compared to AMPH (Hall et al. 2008), and the results presented here lend support to this conclusion.

One potential explanation for the interference with PIT is that the expression of instrumental behavior was compromised by psychostimulant-administration. However, since we did not observe any reduction in activity on the instrumental reminder session that occurred between Pavlovian approach training and PIT testing, it is unlikely explanation. Another possibility is that during the PIT test, competition may have occurred between instrumental and approach behavior and this in turn would result in a

decreased transfer effect (Delameter and Oakeshott 2007; Crombag et al. 2008). If psychostimulant-treated groups were preferentially entering the trough as opposed to performing instrumental behavior, Pavlovian approach ratios or number of trough entries would be expected to be increased. However, our data also do not support this theory as Pavlovian approach ratios were relatively equal between groups. The only significant change in Pavlovian approach was in the group treated with 1.0 mg/kg METH, where there was actually a decrease in the approach ratio. Moreover, there were no differences in number of entries during the CS. Collectively, these results indicate that the administration of METH and AMPH following Pavlovian approach sessions interfered solely with the transfer effect, and did not interfere with the ability to perform instrumental behavior or increase preference for trough entries.

Another plausible explanation for the observed interference effect relates to the timing of the PIT test in regards to drug exposure. Even though there were only a total of three drug administrations, it is possible that the time course of repeated, intermittent administration of psychostimulant utilized here resulted in sensitization. Evidence for this lies in the fact that relatively few administrations, as few as a single dose of 5 mg/kg AMPH, are sufficient to elicit long-term behavioral and neurochemical sensitization (Vanderschuren et al. 1999). Additionally, in the several days following sensitization there is evidence of decreased DA transmission within the NAc which, as time passes, eventually increases and exceeds original baseline DA levels (Segal and Kuzenski 1992a,b). In the present study, PIT testing occurred only 72 hours after the final psychostimulant administration, and it is possible that there was depletion of DA in the NAc due to the repeated AMPH- or METH-administration, particularly at the highest

doses where we observed the greatest decreases. This depletion could result in the interference with PIT since DA transmission within the NAc is critical for expression of PIT (Wyvell and Berridge 2000; Pecina et al. 2006; Lex and Hauber 2008).

In summary, the results presented indicate that the incentive value of the CS is diminished during the PIT test when psychostimulants are administered following Pavlovian approach training. This result was most likely not due to increased Pavlovian approach behavior or general interference with instrumental behavior, but is possibly due to timing of drug administration in relation to PIT testing. Additionally, METH appeared to have a greater effect on PIT than AMPH, supporting previous indications from our lab that METH may affect associative learning to a greater degree than AMPH (Hall et al. 2008). Due to the increasing evidence of the influence that drugs of abuse have on PIT, and since drug-associated cues are thought to play a major role in relapse (Field and Cox 2008), PIT has been suggested as a model of how cues may come to drive drug-seeking behavior that may ultimately result in relapse (Dickinson et al. 2000; Everitt et al. 2001; Cardinal and Everitt 2004). Our findings are consistent with the concept of drugs of abuse altering the PIT response, but further research is needed to understand through what exact mechanisms post-Pavlovian approach psychostimulant administration affects PIT.

vi. Figures and Figure legends

Figure 3.1

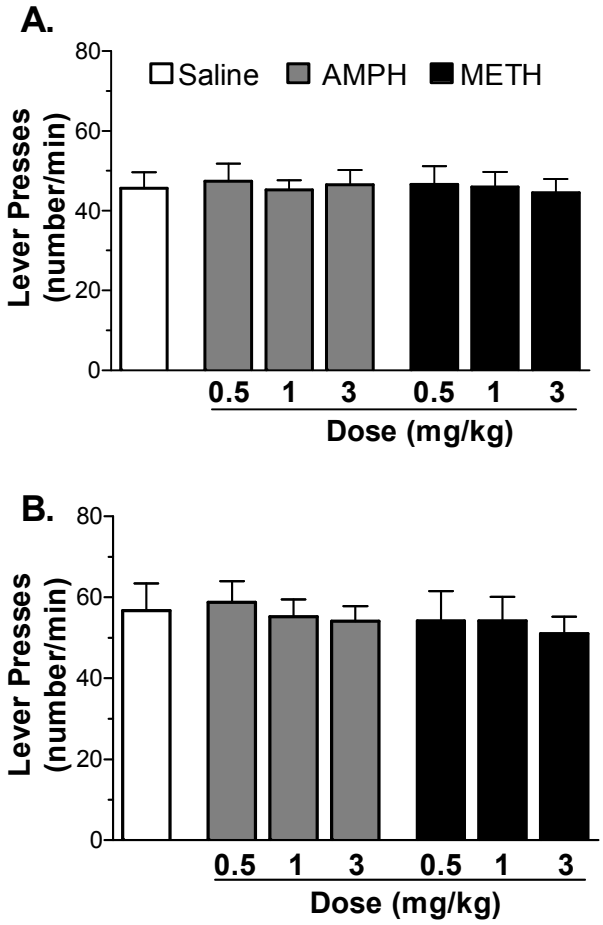

Figure 3.1 Number of lever presses per minute on the final 60 min session of instrumental training (A) and during the 30 min instrumental reminder session that followed Pavlovian approach training (B). Reinforcement in both sessions was on an RR 10 schedule (n = 10/group). Data in this and subsequent figures presented as mean ± SEM.

Figure 3.2.

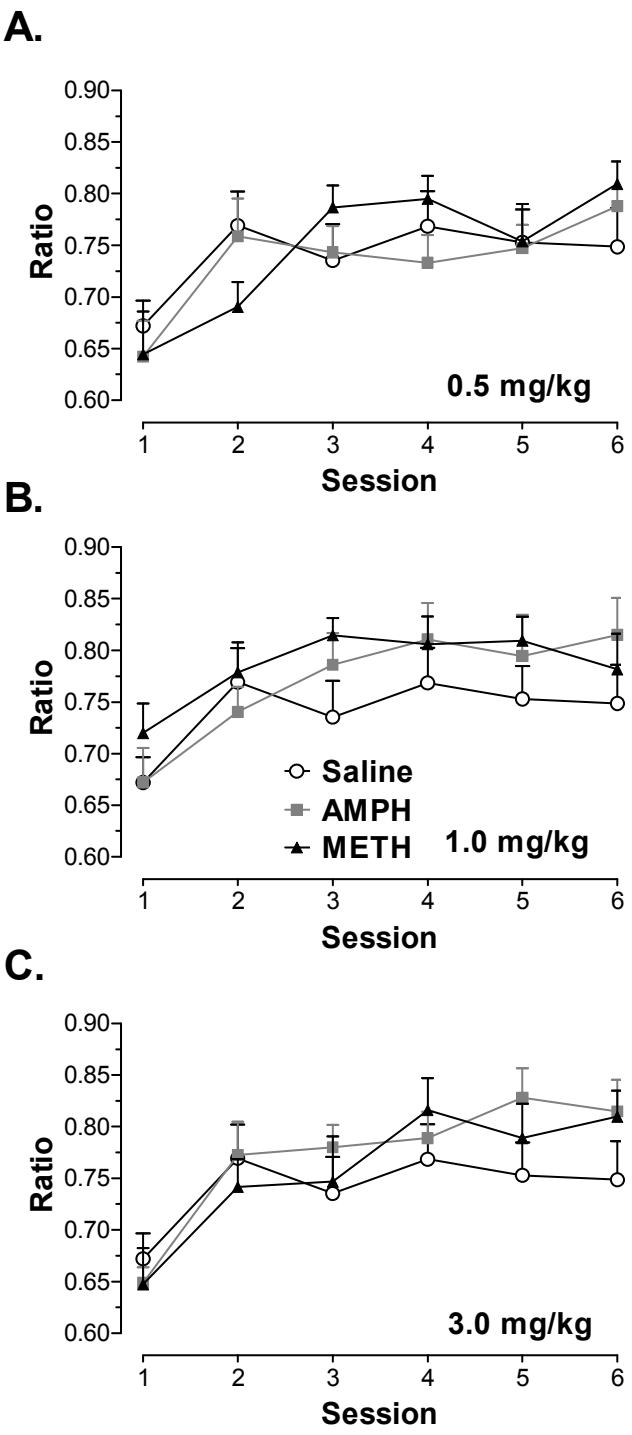

Figure 3.2 Effects of AMPH- or METH-administration at 0.5 mg/kg (A), 1.0 mg/kg (B), or 3.0 mg/kg (C) on Pavlovian approach training (n = 10/group). Drugs were administered immediately post-training on days 1, 3, and 5. Activity is expressed as a ratio of trough entries during the CS compared to 30 s CS time period as plotted across sessions.

Figure 3.3

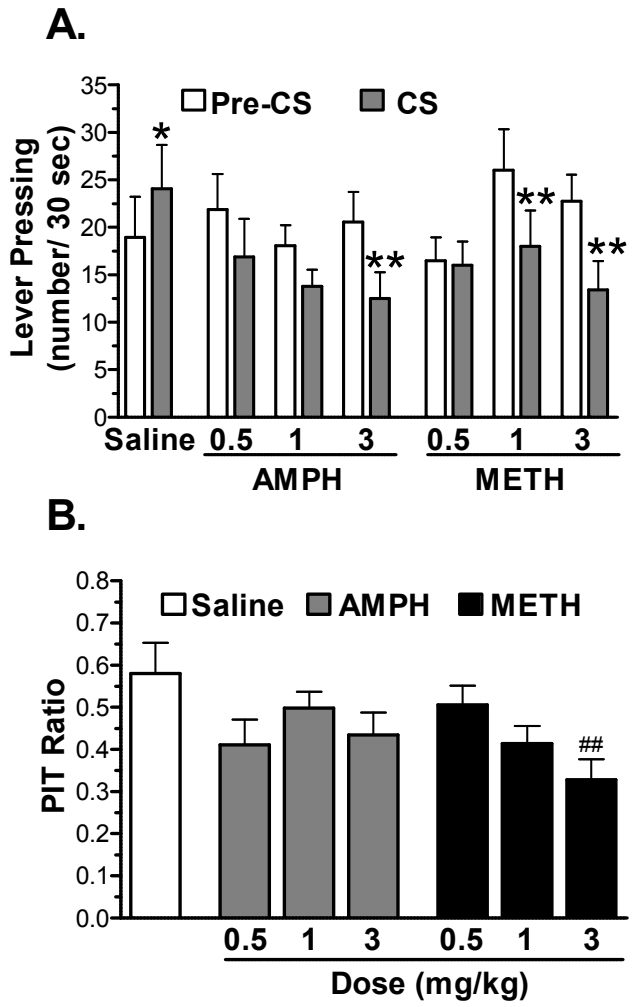

Figure 3.3 Activity on the PIT testing day (n = 10/group). Shown in **A** is rate of lever pressing per 30 sec during pre-CS and CS time periods, * $p < 0.05$, ** $p < 0.01$ compared to CS time period in the same group. Shown in **B** is the transfer effect expressed as a ratio of lever-press responses during CS compared to the 30 s prior to CS onset. $^{\#\#}$ $p < 0.01$ compared to saline.

Figure 3.4

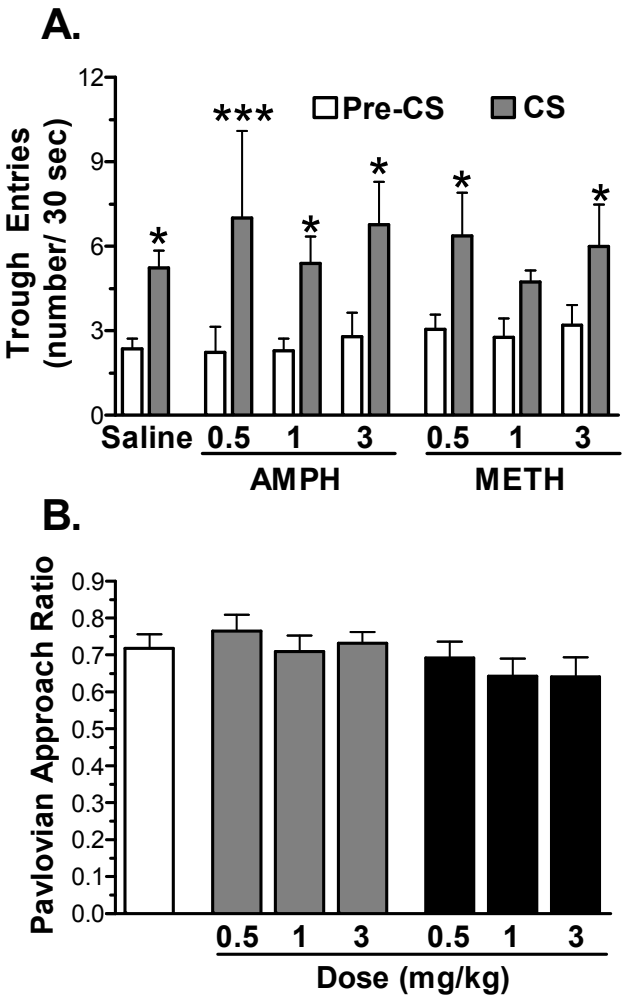

Figure 3.4 Activity on the PIT testing day (n = 10/group). Shown in **A** is rate of trough entries per 30 sec during pre-CS and CS time periods, * $p < 0.05$, *** $p < 0.001$ compared to CS time period in the same group. Shown in **B** is Pavlovian approach expressed as a ratio of trough entries during the CS compared to 30 s pre-CS time period.

Chapter 4.

Experiment 3 – Does glucocorticoid receptor inactivation alter induction or expression of psychostimulant induced sensitization?

i. Abstract

Previous studies have shown that corticosterone (CORT) levels are elevated in rats given psychostimulant drugs such as methamphetamine (METH) and amphetamine (AMPH) and that manipulation of CORT levels alters the locomotor activation these drugs induce. Furthermore, the glucocorticoid receptor (GR) antagonist RU 486 (mifepristone) can decrease the expression of locomotor sensitization induced by repeated exposure to high doses of AMPH. Here, we determined the role of GR receptor activation in both the induction and the expression of sensitization to a moderate dose of AMPH and METH that does not induce stereotypy. Male Sprague-Dawley rats were first habituated to an open-field arena during a 30 min pre-injection and a 60 min post-injection session where saline was administered. They were then divided randomly into three different groups: Control, Induction, and Expression. For the next five daily sessions, rats in the control and expression groups were injected with vehicle (1 ml/kg) 15 min prior to being placed in the open-field arena. After 30 min in the arena, they received either METH or AMPH (1 mg/kg) and were returned to the arena for 60 min. Rats in the induction groups underwent the same procedure but were given 20 mg/kg RU 486 prior to METH or AMPH injection. Following a three-day period of no injections, all rats were challenged with METH or AMPH (1 mg/kg). This was preceded by an injection of vehicle (Control and Induction groups) or 20 mg/kg RU 486 (Expression groups). Compared to the Control group, AMPH-induced sensitization was found to be attenuated

only in the Expression group. These results indicate that GR activation is necessary for expression but not induction of AMPH-induced sensitization. During the challenge session with METH, rats in both the Induction and Expression groups exhibited locomotor sensitization that did not differ in magnitude from that seen in the Control group. In these rats, however, RU 486 increased the number of entries into the center portion of the open-field arena. This effect was observed in both groups, but statistically significant only in the Induction group. Thus, RU 486 treatment led to a change in the pattern, but not overall level, of METH-induced activity. Since increased entry into the center of an open-field arena has been previously associated with decreases in stress and/or anxiety, our findings also suggest that METH-induced stress is decreased in rats whose GRs are blocked during induction of sensitization.

ii. Introduction

Stress causes activation of the hypothalamic-pituitary-adrenal (HPA) axis, which results in release of glucocorticoid hormones from the adrenal glands. In humans, various forms of stress have been correlated with an increase in susceptibility to drug abuse, the development of addiction, and relapse of addictive behaviors (Dembo et al. 1988; Harrison et al. 1997; Koob and Le Moal 1997; Jose et al. 2000). Stress and the associated changes in glucocorticoids have also been shown to have a profound impact on behavior in animal models of drug use and abuse. For example, a variety of stressors, including social isolation, tail pinch, and prenatal stress, can increase the rate of acquisition of cocaine or amphetamine (AMPH) self-administration (Schenk et al. 1987; Bozarth et al. 1989; Piazza et al. 1990; Deminiere et al. 1992). Removal of circulating

glucocorticoids via adrenalectomy decreases acquisition of cocaine self-administration (Goeders and Guerin 1996; Deroche et al. 1997), whereas subsequent administration of corticosterone (CORT) dose-dependently reverses this effect (Deroche et al. 1997). In animal models of relapse, exposure to footshock stress can result in reinstatement of cocaine self-administration (Erb et al. 1996; Buffalari and See 2009) and exposure to footshock, forced swim, or restraint stress can result in reinstatement of previously conditioned place preference (Lu et al. 2002; Sanchez et al. 2003; Kreibich and Blendy 2004; Redila and Chavkin 2008) following extinction. Glucocorticoids also facilitate psychostimulant-induced locomotor activity: adrenalectomy can reduce the acute, locomotor activating effects of both cocaine and amphetamine (AMPH; Cador et al. 1993; Mormede et al. 1994; Marinelli 1997) and this effect can be reversed by administration of CORT (Cador et al. 1993; Marinelli et al. 1997).

Repeated exposure to psychostimulants results in sensitization to their locomotor activating effects. This occurs through a process characterized by two temporally distinct phases that are associated with dissociable, but somewhat overlapping, cellular events (Pierce and Kalivas 1997; Vanderschuren and Kalivas 2000; Vezina 2003). Induction refers to the period during which repeated drug exposure occurs and is accompanied by transient changes that eventually lead to the alterations that underlie the long-term and relatively stable augmentations in behavior. Dopaminergic neurotransmission within the mesolimbic dopamine (DA) pathway and its targets is critical to development of locomotor sensitization (Robinson and Berridge 2000; Steketee 2003). Expression follows induction and is the period when enhancements in behavioral responses can be observed (Robinson and Becker 1986). Stress-related hormones have been shown to be

involved in both stages of the sensitization process. For example, adrenalectomy blocks both the induction and expression of cocaine-induced sensitization (Prasad et al. 1996; Przegalinski et al. 2000; deJong et al. 2009). Conversely, activating the HPA axis through pre-exposure to a stressor such as a tail pinch, footshock, or restraint leads to a sensitized response to cocaine despite no previous exposure to the drug (Antelman 1980; Robinson et al. 1985; Sorg and Kalivas 1991; Sorg 1992). In addition, the effect of food restriction-induced sensitization to cocaine in mice can be ameliorated by preventing the synthesis of glucocorticoids (Rougé-Pont et al. 2005).

Interestingly, studies of the role of glucocorticoids in AMPH-induced locomotor sensitization have provided less consistent results. For example, adrenalectomy has been reported to have no effect (Badiani et al. 1995) or even decrease (Rivet et al. 1989) sensitization to AMPH. The glucocorticoid receptor (GR) antagonist RU 486 can decrease locomotor activity following an AMPH challenge after exposure to a sensitizing regimen of AMPH (De Vries et al. 1996). Furthermore, AMPH also seems to differ from methamphetamine (METH) in its dependence on GR activation for acute locomotor activity, with GR inactivation decreasing acute METH-induced, but not AMPH-induced, locomotion (De Vries et al. 1996; Ago et al. 2009). The experiment described here was undertaken to accomplish two goals. The first was to examine whether inactivation of GRs prevents induction or solely expression of sensitization. Second, with abuse of AMPH and METH spreading (Romanelli et al. 2006), and implications that AMPH is differentially affected by GR activation, it is important to understand what mechanisms mediate the behaviors driven by these two drugs. These goals were achieved by inactivating GRs by administering the GR antagonist RU 486 to Sprague-Dawley rats

during induction or prior to expression of sensitization to AMPH or METH. A moderate (1 mg/kg) dose of AMPH and METH was chosen since it has been demonstrated that this dose is behaviorally activating but does not induce significant levels of stereotypy after repeated administration (Hall et al. 2008).

iii. Materials and Methods

Animals. Male Sprague-Dawley rats (n = 48), 2.5-3.5 months old, were obtained from Harlan (Indianapolis, IN) or bred in our animal facility from Harlan stock rats. They were housed individually, allowed free access to food and water and were kept on a 12:12-h light/dark cycle (lights on at 0800). Before starting experiments, which were performed during the light cycle, all rats were handled for 15 min on five separate occasions. Experimental procedures were approved by the Institutional Animal Care and Use Committee at the University of Illinois and were consistent with the Principles of Laboratory Animal Care (NIH Publication no. 85-23).

Testing Procedures. Activity was measured in an open-field arena (Coulbourn Instruments; Allentown, PA) consisting of an acrylic box (40.6 × 40.6 × 40.6 cm) fitted with two photobeam frames (16 beams/dimension; 2.5 cm between beams). The lower frame (2.5 cm above the arena floor) recorded horizontal activity (i.e., locomotion), whereas the upper frame (15 cm above the floor) recorded vertical activity (i.e. rearing). Each chamber was located in a sound-attenuating cubicle (76 × 80 × 63 cm) that had a 76-mm speaker mounted on the inside wall, and a ceiling-mounted camera between two white lights (4 W each). The speaker was not used in the experiments described here.

Each chamber was connected to a computer running software (TruScan, Coulbourn Instruments) that recorded beam breaks (100 ms sampling rate).

On the first day of the experiment, rats were moved from the colony to the testing room, where they remained in their home cage for a 30-min acclimation period. Fifteen min into the acclimation period all rats received a subcutaneous (s.c.) injection of vehicle. They were then placed individually in the open-field chamber for 30 min, removed and injected with saline (1 ml/kg, i.p.), and returned to the open-field for 60 min. They were subsequently returned to the colony and randomly assigned to one of six groups (n = 8/group): those given intraperitoneal (i.p.) injections of 1.0 mg/kg METH or AMPH plus s.c. injections of vehicle (Control groups), those given METH or AMPH plus RU 486 on each of the treatment days prior to the withdrawal period (Induction groups), and those given METH or AMPH plus RU 486 on the drug challenge day (Expression groups).

On day 2 of the experiment, rats were given a 15 min acclimation period in the open-field and those in the Control and Expression groups were then injected with vehicle; those in the Induction groups were given 20 mg/kg RU 486. After an additional 30 min in the arena, all rats were given a dose of 1.0 mg/kg AMPH or METH. For the next four days (days 3-6), this procedure was repeated such that rats received five injection combinations on successive days. On day 10, which followed a three-day withdrawal period where rats remained in their home cages in the colony room; all rats received the same stimulant drug they had been exposed to previously. In addition, rats in the Control and Induction groups received an injection of vehicle and rats in the Expression groups received 20 mg/kg RU 486. The vehicle and RU 486 injections were given in the same manner as on previous treatment days (i.e., 30 min before stimulant injections).

Data Analysis. Locomotion was calculated as consecutive beam breaks in the lower frame (distance traveled, in cm); rearing was calculated as number of beam breaks in the upper frame. Center entries were quantified based on the number of times the rat moved from the periphery and into the center of the open-field arena. The periphery was defined as the region within 6.25 cm of the walls; thus, the center included a 29 cm^2 area.

Measures of activity were summed in either 5-min bins for both pre- and post-injection time periods for locomotion, or in 60-min post-injection time periods for center-entries and rearing activity. The presence of locomotor sensitization was determined by comparing distance traveled on test days 2 (first exposure) and 10 (challenge) and was analyzed using two-factor repeated measure ANOVAs (day x time). The influence of drug and treatment type on the first exposure and challenge sessions was analyzed via two-factor ANOVAs (drug x treatment) for rearing and center entries. Analysis of rearing sensitization was performed by comparing activity on test days 2 and 10 with a mixed two-factor ANOVA (group x day, with day as the repeated measure). Whenever appropriate, post-hoc comparisons of specific groups were done with Tukey tests.

Drugs. *d*-Amphetamine sulfate and *d*-methamphetamine HCl (Sigma-Aldrich; St. Louis, MO) were dissolved in sterile saline at concentrations of 1.0 mg/ml and injected at a volume of 1 ml/kg. Dosages were calculated as the weight of the salt. RU 486 (Caymen Chemicals, St. Petersburg, FL) was dissolved in vehicle (propylene glycol) at a concentration of 20 mg/kg and injected at a volume of 1 ml/kg. This dose was chosen based on previous reports showing its ability to alter AMPH-induced behavior in an open-field arena (DeVries et al. 1996).

iv. Results

The effects of GR inactivation on AMPH- and METH-induced sensitization are shown in Fig 4.1. Comparison of activity on the first exposure and challenge test sessions revealed modest levels of sensitization in Control groups (Figs. 4.1A and 1B), with both AMPH- and METH-treated rats exhibiting non-significant trends toward a main effect of day (AMPH: $F_{1,287} = 4.94$, $p = 0.062$; METH: $F_{1, 287} = 3.95$, $p = 0.087$), significant main effects of time (AMPH: $F_{17, 287} = 25.6$, $p < 0.001$; METH: $F_{17, 287} = 4.71$), and a significant interaction effect only in the METH-treated group ($F_{17, 287} = 4.71$, $p < 0.001$). Post-hoc analysis in this group revealed that METH-induced sensitization of locomotion occurred primarily in the first 30 min following injection (Fig 4.1B). GR inactivation had no significant effect on the induction of AMPH- or METH-induced sensitization (Figs. 4.1C and D). For both of these groups, there were main effects of day (AMPH: $F_{1,287} = 13.7$, $p < 0.01$; METH: $F_{1, 287} = 27.7$, $p < 0.01$), time (AMPH: $F_{17,287} = 24.2$, $p < 0.001$; METH: $F_{17, 287} = 84.1$, $p < 0.001$), and day x time interactions (AMPH: $F_{17,287} = 3.08$, $p < 0.001$; METH: $F_{17, 287} = 5.11$, $p < 0.001$). GR inactivation solely on the challenge day resulted in no measurable levels of sensitization in the AMPH Expression group (Fig 4.1E), with analysis revealing no significant main effect of day or interaction effects, and only a main effect of time ($F_{17, 287} = 37.6$, $p < 0.001$). Robust sensitization was present in the METH Expression group however, with significant main effect of day ($F_{1, 287} = 16.4$, $p < 0.01$) time ($F_{17, 287} = 52.3$, $p < 0.001$), and interaction effects ($F_{17, 287} = 3.73$, $p < 0.001$).

Examination of the number of center entries following the first exposure to AMPH or METH revealed no significant effect of GR inactivation in either

psychostimulant-treated group. The only statistically significant effect was a main effect of drug ($F_{1,47} = 7.18$, $p < 0.05$; Fig 4.2A). However, during the challenge session, RU 486 had differential effects in AMPH- compared to METH-induced treated groups. ANOVA revealed significant main effects of drug ($F_{1,28} = 25.3$, $p < 0.001$) and group ($F_{2,28} = 4.26$, $p < 0.05$), along with a drug x group interaction ($F_{2,28} = 3.42$, $p < 0.05$). In the METH-treated Induction group, repeated administration of RU 486 prior to METH injections significantly increased the number of center entries made during the challenge session when no RU 486 was given. Injection of RU 486 before METH challenge (Expression group) also tended to increase center entries, but this was not significantly different from controls (Fig 4.2B). Entries made by rats in the Induction and Expression groups given METH were also significantly increased over those made by the same groups given AMPH (Fig 4.2B). However, there were no differences in number of entries between METH and AMPH groups given vehicle, and center entries in the AMPH-treated groups were not altered in either the Induction or Expression groups compared to the Control group.

Examination of rearing following acute (Day 2) AMPH- or METH-administration revealed no statistically significant effects of RU 486 on rearing activity, with only a significant effect of drug present ($F_{1,47} = 7.18$, $p < 0.05$: Fig. 4.3A). Similar to the acute treatment day, rearing following AMPH or METH challenge was unaffected by RU 486 treatment (Fig 4.3B). The presence of rearing sensitization was determined using a mixed two-factor (group x day) by comparing acute and challenge days, with only the main effect of day ($F_{5, 95} = 5.85$, $p < 0.05$) found to be statistically significant.

v. Discussion

The results found here suggest differing roles for GR activation in METH- and AMPH-induced locomotor sensitization. First, sensitization of locomotion was observed in all METH-treated groups, but only AMPH-treated animals pre-treated with vehicle and those given RU 486 before daily AMPH injections exhibited sensitized locomotion following AMPH challenge. Thus, RU 486 blocked the expression of AMPH-induced sensitization when it was given before the AMPH challenge. This result is consistent with a previous report showing that this same dose of RU 486 decreased the expression of sensitization induced by 3.0 mg/kg AMPH (De Vries et al. 1996). Additionally, these results seem to indicate that levels of AMPH-induced locomotor activity are influenced to a greater extent by GR inactivation than levels of METH-induced locomotor activity. There is evidence in the literature for dissociation between locomotor activity and neurotransmitter levels. Administration of similar doses of METH and AMPH can result differing levels of neurotransmitter release in certain brain areas such as the mPFC and hippocampus (Leonard 1972; Kuczenski et al. 1995; Shoblock et al. 2003), but those same doses can induce similar levels of activity (Kuczenski et al. 1995, Shoblock et al. 2003). Perhaps levels of AMPH-induced activity and neurotransmitter levels are more sensitive to perturbations in GR activity, while METH-induced locomotion at this dose is not.

Locomotor sensitization at this dose has been described before (Tilson and Rech 1973; Bevins and Peterson 2004; Hall et al. 2008), but an examination of the contribution of GR activation to development of sensitization has not previously been characterized. Taken together, these results indicate that GR activation is necessary for expression, but

not induction of AMPH-induced sensitization. This effect is likely predicated by the interaction between GRs and DA transmission since DA transmission within this region is critical for locomotor sensitization (Robinson and Berridge 2000; Steketee 2003) and there is mounting evidence for a role in GRs in regulating DA activity within the mesolimbic dopaminergic pathway. For instance, GRs are present in ventral tegmental area DA containing cells (Baxter and Rousseau 1979). Activating GRs on these cells via dexamethasone, a synthetic glucocorticoid, can potentiate the synaptic strength enhanced by a stressful event, conversely inactivation of GRs using RU 486 can abolish this effect (Saal et al. 2003). Additionally, inactivating GRs in the VTA using RU 486 can decrease DA efflux dependent upon time within the circadian rhythm. Moreover, activating GRs using CORT the nucleus accumbens can potentiate DA efflux regardless (Tye et al. 2009).

We also examined number of entries into the center of the open-field arena since this behavior is often quantified as a means of determining levels of anxiety (Prut and Belzung 2003). When placed in open areas rats tend to prefer to stay toward the walls. This is a behavior known as thigmotaxis, which is thought to indicate a fear response (Walsh and Cummins 1976; Prut and Belzung 2003). Furthermore, anxiolytic compounds increase center activity (Montgomery 1955; Zbinden and Randall 1967; Prut and Belzung 2003). Following first exposure, GR inactivation tended to increase number of center entries in both AMPH- and METH-treated groups indicating an overall reduction in anxiety. However, following the challenge exposure, only METH-treated groups exhibited any decrease in anxiety with the greatest effect observed in the METH Induction group. This is notable since there was no corresponding increase in METH-

induced locomotor activity in the Induction or Expression groups compared to the Control group. It is also notable that this effect was greatest in the Induction group which did not receive GR antagonist prior to the challenge exposure indicating that the anxiogenic properties of repeated METH administration can be reduced by administration of a GR antagonist. Additionally, between-drug analysis revealed no difference in psychostimulant-induced anxiety in the Control groups, with both the METH Induction and Expression group exhibiting significantly less anxiety than similarly treated AMPH groups. Taken together, these results indicate that the anxiogenic properties of METH can be modified without altering locomotor activity. Since stress is thought to be an important predictor of the development and subsequent relapse of addiction-related behaviors (Dembo et al. 1988; Harrison et al. 1997; Koob and Le Moal 1997, Sinha 2008), it is important to understand the how specific stress-related mechanisms modify the drug-response.

Following both acute and repeated psychostimulant administration, rearing activity was unaffected by the inactivation of GRs with all groups exhibiting relatively similar levels of activity. It has been noted before that vertical activity is possibly mediated via overlapping but somewhat distinct mechanisms from horizontal activity (Walsh and Cummins 1975; Crawley et al. 1997). For example, cocaine-induced vertical activity exhibits greater sensitivity to serotonin receptor manipulation, with a 5-HT_{1B} receptor agonist decreasing vertical but not horizontal activity (Przegalinski et al. 2007). Additionally, infusion of DA into the nucleus accumbens shell potentiates vertical activity to a greater extant than horizontal activity (Swanson et al. 1997). Furthermore, previous work from our lab has also noted dissociation between horizontal and vertical

activity (Hall et al. 2008; Hall et al. 2009). Based upon the fact that here we found AMPH-induced locomotion in the Expression group was decreased, but rearing behavior remained unaffected, one of the possible mechanisms mediating these differences may be related to activation of GRs.

In summary, the results of this study suggest that GR activation is necessary for the expression, but not the induction, of AMPH-induced locomotor sensitization. In contrast, GR inactivation had relatively little influence on METH-induced locomotor sensitization. However, METH-treated groups experienced a greater decrease in anxiety-related behaviors. Thus, METH-sensitized rats still underwent sensitization of locomotion, but experienced a decrease in the anxiogenic properties of repeated METH administration when GR activation was blocked during the induction phase. Taken together it would seem that METH-induced sensitization of locomotion occurs independently of GR activation. These findings are interesting in light of the significant effect stress-related hormones have on drug-induced behaviors (Dembo et al. 1988; Harrison et al. 1997; Koob and Le Moal 1997; Jose et al. 2000; Sinha 2008). Furthermore, understanding where psychostimulants diverge in their actions provides valuable information that could eventually have bearing upon drug-specific treatment of addiction.

vi. Figures and Figure legends

Figure 4.1

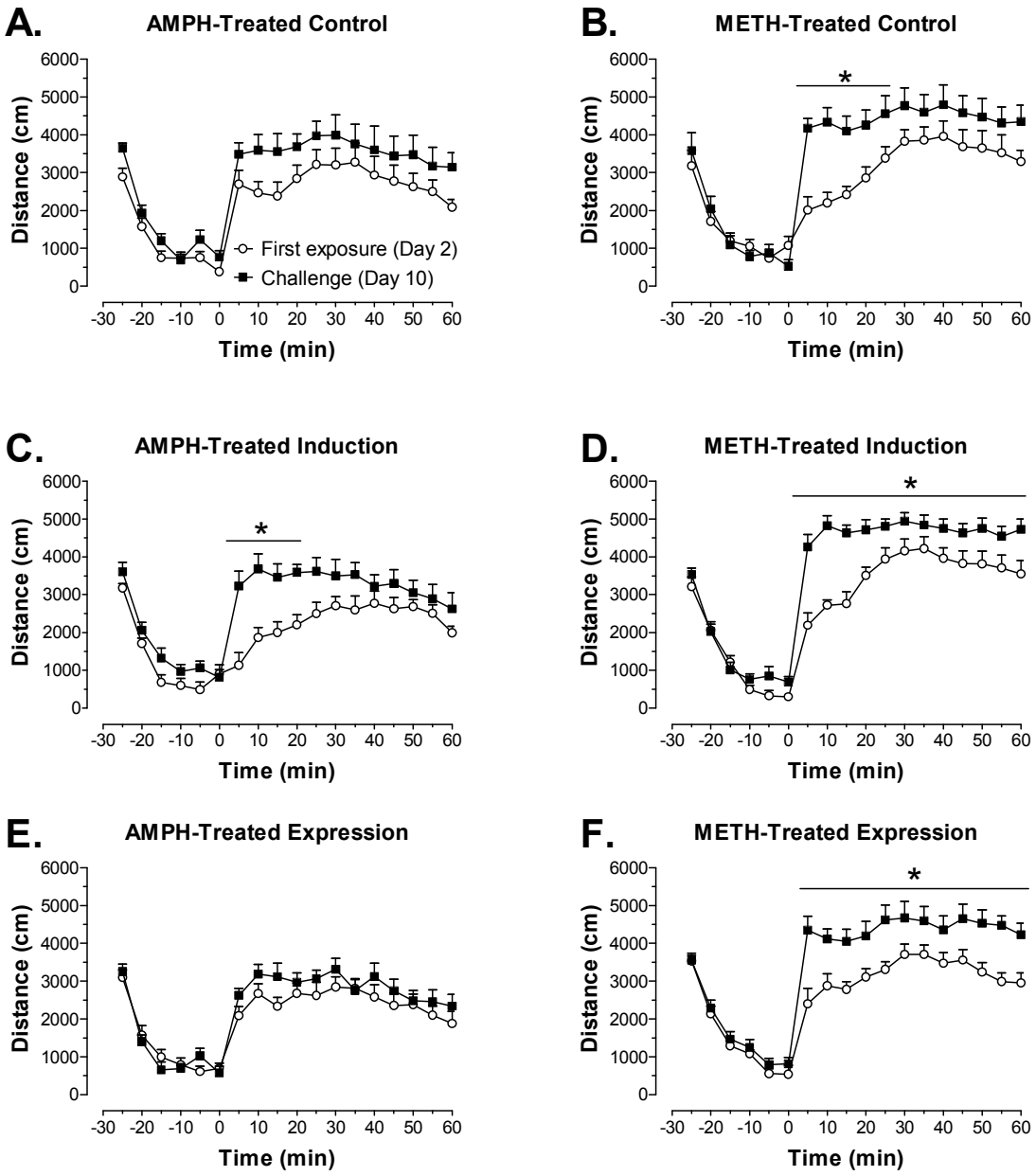

Figure 4.1 The effects of 20 mg/kg RU 486 on locomotor activity following the first exposure or challenge with 1.0 mg/kg AMPH or METH (n = 8 rats/group). RU 486 was administered either repeatedly during days 2-6 (Induction groups) or only on the challenge session that occurred on day 10 (Expression groups). Control groups were given injections of vehicle before every AMPH or METH injection. RU 486 or vehicle was given 45 min before psychostimulant injections. Shown is the ambulatory activity (mean ± SEM) before and after psychostimulant injection (at time = 0). *$p < 0.05$ compared to the same time period on Day 2.

Figure 4.2

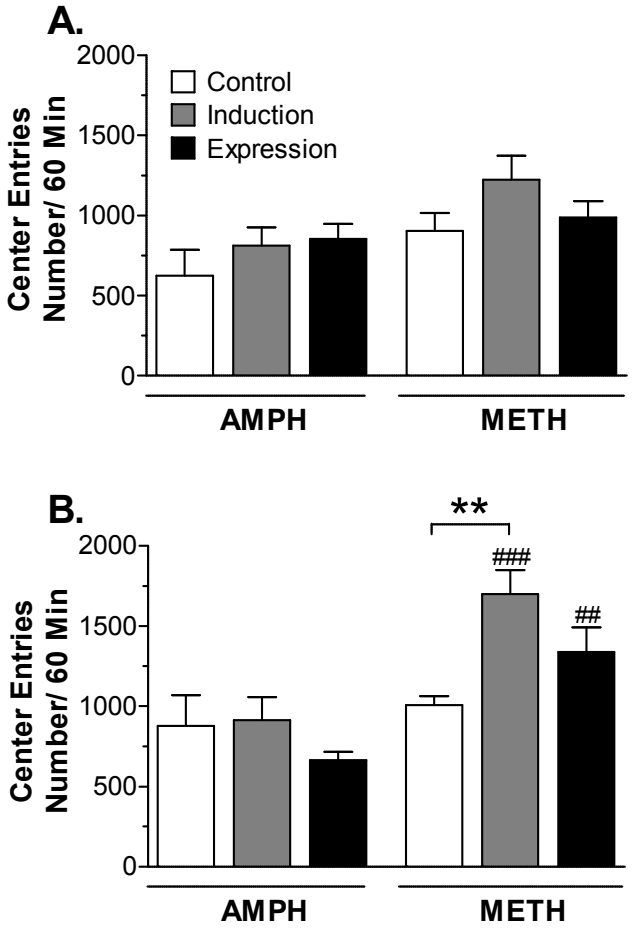

Figure 4.2 The effects of 20 mg/kg RU 486 on entries into the center of the open-field arena after the first exposure (A) and challenge (B) with 1.0 mg/kg AMPH or METH (n = 8 rats/group). Shown is the total number of center entries during the 60-min period (mean ± SEM) following psychostimulant injection in the groups described in Fig. 1. $^{**}p < 0.01$; $^{\#\#\#}p < 0.001$ and $^{\#\#}p < 0.01$, compared to the Induction and Expression groups given AMPH, respectively

Figure 4.3

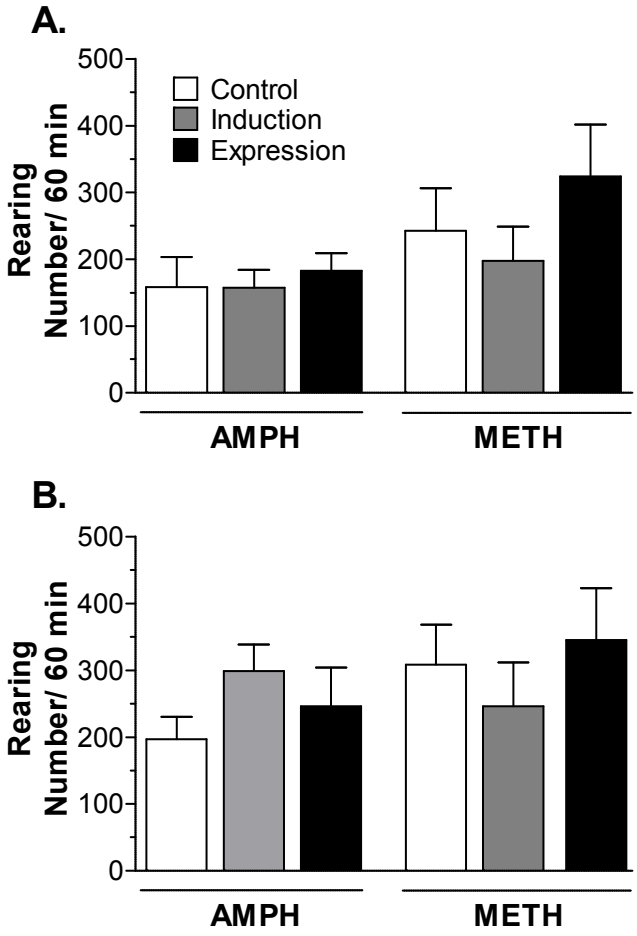

Figure 4.3 The effects of 20 mg/kg RU 486 on rearing activity after the first exposure (A) and challenge (B) with 1.0 mg/kg AMPH or METH (n = 8 rats/group). Shown is the total number of rears (i.e., photobeam breaks in the vertical plane) during the 60-min period (mean ± SEM) following psychostimulant injection in the groups described in Fig. 1.

Chapter 5.

Experiment 4 – What is the contribution of D1 receptor activation in the medial prefrontal cortex to psychostimulant induced locomotor activity?[2]

i. Abstract

The medial prefrontal cortex (mPFC) is a component of the mesolimbic dopamine (DA) system involved in psychostimulant-induced hyperactivity and previous studies have shown that altering DA transmission or D2 receptors within the mPFC can decrease this stimulant effect. The goal of this study was to investigate a potential modulatory role for D1 receptors in the mPFC in amphetamine (AMPH)- and methamphetamine (METH)-induced hyperactivity. Locomotor activity in an open-field arena was measured in male, Sprague-Dawley rats given an intra-mPFC infusion of vehicle or the D1 receptor antagonist SCH 23390 (0.25 or 1.0 μg) prior to systemic (i.p.) injection of saline, AMPH (1 mg/kg) or METH (1 mg/kg). We found that SCH 23390 produced a dose-dependent decrease in AMPH- and METH-induced locomotion and rearing, but had no significant effect on spontaneous behavior that occurred following systemic saline injections. Because SCH 23390 has been shown to have agonist-like properties at 5-HT_{2C} receptors, a follow-up experiment was performed to determine if this contributed to the attenuation of METH-induced activity that we observed. Rats were given intra-mPFC infusions of both SCH 23390 (1.0 μg) and the 5-HT_{2C} antagonist RS 102221 (0.25 μg) prior to METH (1 mg/kg, i.p.). The addition of the 5-HT_{2C} antagonist failed to alter SCH 23390-induced decreases in METH-induced locomotion and rearing; infusion of RS 102221 alone had no

[2] This work was previously published and is reproduced here with permission from the copyright holder Springer. Bibliographic information: Hall DA, Powers JP, Gulley JM (2009) Blockade of dopamine D1 receptors in the medial prefrontal cortex attenuates amphetamine- and methamphetamine-induced locomotor acitivity in the rat. Brain Res. 1300: 1-7

significant effects on locomotion and produced a non-significant decrease in rearing. The results of these studies suggest that D1 activation in the mPFC plays a significant role in AMPH- and METH-induced hyperactivity.

ii. Introduction

The mesolimbic dopamine (DA) system plays a critical role in psychostimulant-induced locomotor activity. One component of this circuit, the medial prefrontal cortex (mPFC), receives DA input from the ventral tegmental area (VTA) and contains both D1 and D2 receptors. Evidence from several studies suggests that D1 receptors are primarily localized on GABAergic interneurons and, to a lesser degree, on pyramidal excitatory amino acid neurons, whereas D2 receptors tend to be localized primarily on pyramidal cells and to a lesser degree on GABAergic interneurons (Al-Tikriti et al. 1992; Vincent et al. 1993; Vincent et al. 1995). There is extensive experimental evidence for the importance of DA transmission within the mPFC in the behavioral response to psychostimulants. For example, amphetamine (AMPH) dose-dependently increases extracellular levels of DA within the mPFC (Moghaddam and Bunney 1989; Maisonneuve et al. 1990). Depletion of mPFC DA via local infusion of the neurotoxin 6-OHDA has been shown to prevent the hyperactivity induced by a single administration of AMPH (Dunnett et al. 1984; Banks and Gratton 1995; King and Finlay 1995) as well as the development of behavioral sensitization following repeated exposure to AMPH (Bjijou et al. 2002). These findings are not unequivocal however, as at least one study demonstrated an increase in the sensitized response to AMPH following mPFC DA depletion (Banks and Gratton 1995). Furthermore, motor stereotypies induced by

relatively high doses of AMPH (2.5-10 mg/kg) are also reported to be increased by these lesions (Carter and Pycock 1980; Sokolowski and Salamone 1993; Espejo and Minano 1995). The reason for these disparate findings is not clear, but one contributing factor may be related to compensatory changes in the function of D1 and D2 DA receptors that occur following lesion-induced depletions of endogenous DA concentrations.

A clear role for DA receptors within the mPFC has been demonstrated in a number of studies that utilized pharmacological manipulations of psychostimulant-induced behavior. For example, intra-mPFC administration of the non-selective D1/D2 antagonist flupenthixol decreases acute AMPH-induced locomotion (Bast et al. 2002). Interestingly, intra-mPFC infusion of the selective D2 receptor agonist quinpirole decreases both acute and sensitized cocaine-induced activity (Beyer and Steketee 2000; Beyer and Steketee 2002) and prevents expression of neurochemical sensitization by attenuating cocaine-induced increases in DA (Beyer and Steketee 2002). Lastly, when compared to wild-type controls, D2 receptor knockout mice do not exhibit locomotor stereotypy after repeated injections of a high dose (5 mg/kg) of METH (Glickstein and Schmauss 2004).

In contrast to the number of studies assessing the role of D2 receptor activation in the mPFC in psychostimulant-induced locomotion, considerably less attention has been paid to the role of D1 receptor activation. Thus, the present study examined the contribution of D1 receptor activation in the mPFC to AMPH- and methamphetamine (METH)-induced locomotor activity. To accomplish this, we recorded open-field behavior in rats given intra-mPFC infusions of the D1 antagonist SCH 23390 just before they received a systemic injection of a dose of AMPH or METH (1 mg/kg, i.p.) that

increases locomotion and rearing without producing motor stereotypies (Hall et al., 2008). Because previous studies demonstrated that SCH 23390 has agonist-like effects at 5-HT_{2C} receptors (Briggs et al. 1991) and that this activity is responsible for its ability to decrease 3,4-methylenedioxymethamphetamine (MDMA)-induced activity and locomotor sensitization (Ramos et al. 2005), we also tested the effects of intra-mPFC infusion of the 5-HT_{2C} receptor antagonist RS 102221, alone and in combination with SCH 23390, on METH-induced behavior. These latter experiments were performed only in METH- treated rats since initial examination of METH- and AMPH-induced locomotor activity after infusion of either dose of SCH 23390 revealed no significant between-group differences.

iii. Materials and Methods

Animals. Male Sprague–Dawley rats (n = 77), 2.5–3.5 months old, were obtained from Harlan (Indianapolis, IN) or bred in our animal facility from Harlan stock rats. They were housed individually, allowed free access to food and water and were kept on a 12:12-h light/dark cycle (lights on at 0800). Before starting experiments, which were performed during the light cycle, rats were handled for 15 min on five separate occasions. Experimental procedures were approved by the Institutional Animal Care and Use Committee at the University of Illinois and were consistent with the Principles of Laboratory Animal Care (NIH Publication no. 85-23).

Surgical Procedures. In preparation for bilateral cannulae implantation, rats were given atropine (0.1 mg/kg, i.p.) and anaesthetized with a combination of ketamine (90 mg/kg, i.m.) and xylaxine (10 mg/kg, i.m.). They were then placed in a stereotaxic frame and a

small incision was made to expose the skull. A high speed microdrill was then used to drill holes overlying the left and right mPFC (+3.0 mm AP and ± 1.2 mm ML from bregma; Paxinos and Watson, 1997) and a 28-G guide cannulae (C313G; Plastics One, Roanoke, VA) was lowered 3.2 mm below the skull surface. Three additional holes were drilled in the skull for insertion of stainless-steel support screws, and dental acrylic was added to cement the cannulae in place. Dummy cannulae cut to the same length as the guide cannulae (C313DC, Plastics One) were then inserted to prevent obstruction. Lastly, rats were administered carprofen (5 mg/kg, s.c.) at 12-24 hour intervals for one day post-surgery and were allowed at least seven days for recovery before the start of experiments.

Drugs. SCH 23390 ((R)-(þ)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride) and RS 102221 (8-[5-(2,4-dimethoxy-5-(4-trifluoromethylphenylsulfonamido)phenyl-5-oxopentyl]-1,3,8-triazaspiro[4,5]decane-2,4-dione hydrochloride) were obtained from Fisher Scientific (St. Louis, MO). SCH 23390 was dissolved in 0.9% saline and RS 10221 was dissolved in 10% dimethyl sulfoxide in 0.9% sterile saline. Dosages of *d*-AMPH sulfate and *d*-METH HCl (Sigma-Aldrich; St. Louis, MO) were calculated as the weight of the salt, dissolved in 0.9% sterile saline and injected at a volume of 1 ml/kg.

Test Procedures. Activity was measured in open-field arenas consisting of an acrylic box (40.6×40.6×40.6 cm) fitted with two photobeam frames (16 beams/dimension; 2.5 cm between beams; Coulbourn Instruments; Allentown, PA). The lower frame (2.5 cm above the arena floor) recorded horizontal activity (i.e., locomotion), whereas the upper frame (15 cm above the floor) recorded vertical activity (i.e. rearing). Each chamber was

located in a sound-attenuating cubicle (76×80×63 cm) that had a 76-mm speaker mounted on the inside wall, and a ceiling-mounted camera between two white lights (4 W each). The speaker was not used in the experiments described here. Each chamber was connected to a computer running software (TruScan, Coulbourn Instruments) that recorded beam breaks (100 ms sampling rate).

On the first day of the experiment, rats were moved from the colony to the testing room where they remained in their home cage for a 30-min acclimation period. Subsequently, injector cannulae (C313I; Plastics One) that extended 1 mm beyond the guide cannulae were inserted into the mPFC and rats were infused with either vehicle (saline; 0.5 µl/side; n = 48) or SCH 23390 (0.25 or 1.0 µg/side; n = 7 and n = 6, respectively). The injector cannulae were attached to flexible plastic tubing that was connected to 10 µL Hamilton syringes containing the appropriate solution for infusion. The syringes were mounted on a syringe pump (model #SP100I, World Precision Instruments; Sarasota, FL) that was programmed to deliver a volume of 0.5 µL/side over a period of 1 min. The cannulae were left in place for 1 min following the infusion to allow for diffusion. Rats were then placed individually into an open-field chamber for 15 min, removed and injected with saline (1 ml/kg, i.p.), and then returned to the open-field for an additional 60 min.

On the second day of the experiment this procedure was repeated with the following exceptions. First, only rats who received intra-mPFC infusions of vehicle on day one were tested on day two. Second, rats tested on day two were randomly assigned to receive an infusion of SCH 23390 alone (0.25 or 1.0 µg/side; n = 7/group and n = 10/group, respectively), RS 10221 alone (0.25 µg/side; n = 8), or co-infusion of SCH

23390 + RS 10221 (1.0 + 0.25 μg/side; n = 8). These doses of SCH 23390 and RS 102221 were chosen based upon their previously reported ability to alter behavior when infused into the mPFC (Runyan and Dash 2004; Sanchez et al. 2003; Ramos et al. 2005). Third, rats were randomly assigned to receive an i.p. injection of 1.0 mg/kg AMPH or 1.0 mg/kg METH, 15 min after they were given their assigned intra-mPFC infusion. The effects of RS 10221 alone or in combination with SCH 23390 were only tested in rats given 1.0 mg/kg METH.

Histology. After completion of the experiment, rats were deeply anesthetized and transcardially perfused with 0.9% saline and 10:1 dilution buffered formalin, and their brains were removed and stored in formalin. They were then sliced on a sliding microtome (60 μm sections), mounted on slides and stained with neutral red. Placements of injector cannulae in the mPFC were then verified via light microscopy by an observer who was unaware of the rat's treatment or behavioral response. Data were not used from rats that had placements determined to be outside the infralimbic or prelimbic regions of the mPFC or whose tissue revealed significant necrosis that was characteristic of infection around the area of the guide cannulae. Shown in Fig. 5.1 is a diagram of the approximate location of injections in the mPFC for the data presented here.

Data Analysis. Locomotion was calculated as consecutive beam breaks in the lower frame (distance, in m); rearing was calculated as number of beam breaks in the upper frame. Activity was summed in 30- or 60-min bins. The influence of intra-mPFC infusions on spontaneous activity (experiment day 1) was analyzed using one-way ANOVA. The influence of infusions on AMPH- and METH-induced activity (experiment day 2) was analyzed with two-way between-factors ANOVA (infusion x

drug). Lastly, the influence of infusions of RS 102221 alone or in combination with SCH 23390 on METH-induced locomotion was analyzed using one-way ANOVA. Pairwise comparisons were done with Tukey post-hoc tests where appropriate.

iv. Results

Effects of SCH 23390 on spontaneous activity

To determine if intra-mPFC infusions of SCH 23390 altered general motor behavior, spontaneous activity was measured during the first 30 min following infusion of vehicle or SCH 23390 (0.25 or 1.0 μg/side). Fifteen min after the infusions, rats were given an i.p. injection of saline. As shown in Fig. 5.2A, we found no significant effect of infusion on locomotion ($F_{2,60} = 1.97$, $p = 0.15$). Although SCH 23390 infusions did tend to decrease rearing, particularly at the 1.0 μg/side dose (Fig. 5.2B), the main effect of infusion was not significant ($F_{2,60} = 2.29$, $p = 0.11$).

Effects of SCH 23390 on AMPH- and METH-induced activity

On experiment day two, rats received either SCH 23390 (0.25 or 1.0 μg/side) or vehicle to examine if the indirect activation of D1 receptors by AMPH or METH contributes to the locomotor activation these drugs produce. A two-way ANOVA (drug x infusion) on the locomotion data revealed main effects of infusion ($F_{2,47} = 13.69$, $p < 0.001$) and drug ($F_{1,47} = 5.60$, $p < 0.05$), but no drug x infusion interaction ($F_{2,47} = 0.12$, $p = 0.89$). A dose-dependent decrease was observed in both AMPH- and METH-induced locomotion, with post-hoc analysis revealing a significant effect at the 1.0 μg/side dose compared to vehicle infused rats (Fig. 5.3A). In METH-treated rats, the level of

locomotor activity at the 1.0 μg/side SCH 23390 dose was also significantly lower than that observed in rats who received 0.25 μg/side SCH 23390 (Fig. 5.3A). Comparison of data from rats infused with vehicle shows that the locomotion induced by the tested dose of AMPH was not significantly different from that induced by METH.

SCH 23390 also produced a dose-dependent decrease in AMPH- and METH-induced rearing. Two-way ANOVA revealed significant main effects of infusion ($F_{2,46} = 8.59$, $p < 0.001$) and drug ($F_{1,46} = 15.0$, $p < 0.001$), and a drug x infusion interaction ($F_{2,46} = 4.17$, $p < 0.05$). SCH 23390 significantly decreased METH-induced rearing at both doses of SCH 23390, with the greatest decrease observed in rats that received 1.0 μg/side. The dose-dependent decrease is also evident in AMPH-treated rats, but this effect was not statistically significant. Comparison of data from rats infused with vehicle shows that METH was more potent at increasing rearing compared to AMPH; however, both doses of SCH 23390 reduced METH-induced rearing to levels comparable to that seen in all three of the AMPH-treated groups (Fig. 5.3B).

Effects of RS 102221 on METH-induced activity

In a group of rats given systemic injections of 1.0 mg/kg METH, we sought to determine if indirect activation of 5-HT_{2C} receptors by METH contributes to METH-induced activity. Furthermore, we tested if SCH 23390's ability to act as an agonist at 5-HT_{2C} receptors was responsible for the reduction in METH-induced activity that we observed following intra-mPFC infusion of 1.0 μg/side SCH 23390. Thus, rats were given intra-mPFC infusions of the 5-HT_{2C} receptor antagonist RS 102221 (0.25 μg/side), alone or in combination with 1.0 μg/side SCH 23390, followed by 1.0 mg/kg METH. As

shown in Fig. 5.4A, RS 102221 had no significant effect on METH-induced locomotion and did not alter the ability of SCH 23390 to reduce METH's effects on locomotion. This was evident by the significant main effect of infusion in a one-way ANOVA ($F_{3,32} = 7.76$, $p < 0.001$). While a similar pattern of results was observed for rearing ($F_{3,32} = 8.12$, $p < 0.001$), it was the case that infusion of RS 102221 tended to reduce METH-induced rearing and enhance the SCH 23390-induced decrease in METH-induced rearing (Fig. 5.4B).

v. Discussion

Whereas the contribution of D2 receptors within the mPFC to psychostimulant-induced locomotor activity has been relatively well characterized, a potential role for D1 receptors has been less studied. To address this, we determined the effects of locally infusing the selective D1 antagonist SCH 23390 into the mPFC on AMPH- and METH-induced locomotor activation. We found this infusion produced a dose dependent decrease in AMPH- and METH- induced locomotion and rearing. This effect did not appear to be due to SCH 23390-induced changes in overall motor behavior because infusion of 0.25 or 1.0 μg/side did not significantly reduce spontaneous locomotion and rearing. Furthermore, previous studies in both rats and mice also reported little to no effect of intra-mPFC infusions of SCH 23390 on motor activity (Vezina et al. 1994; Radcliffe and Erwin 1996). Thus, our results suggest that indirect activation of D1 receptors by AMPH and METH, most likely via elevated concentrations of extracellular DA in the mPFC, is essential for the hyperactivity that these drugs induce.

Earlier studies indirectly implicated D1 receptors in psychostimulant-induced locomotor activity. For example, systemically administered SCH 23390 was reported to decrease both the acute and sensitized response to AMPH (Vezina and Stewart 1989). Mice with genetic deletion of the D1 receptor have been shown to be less sensitive to the locomotor activating effects of both cocaine and AMPH (Zhang et al. 2000). Furthermore, intra-mPFC infusion of the mixed D1/D2 receptor antagonist flupenthixol decreased AMPH-induced activity (Bast et al. 2002), though the authors hypothesized that this was due to inactivation of the D2 receptor. Selectively activating D1 receptors in the mPFC has been shown to have mixed effects on acute psychostimulant-induced activity. For example, an intra-mPFC infusion of the D1 agonist SKF 81297 has been shown to increase acute cocaine-induced vertical activity (Sorg et al. 2001), while an infusion of the D1 partial agonist SKF 38393 produced no significant effect (Beyer and Steketee 2000). Furthermore, infusion of the D1 agonists dihydrexidine or A 68930 into the mPFC resulted in a significant decrease in AMPH-induced hyperactivity (Isaacson et al. 2004). The effects of intra-mPFC infusion of a D1 or D2 agonist on cocaine-induced locomotor sensitization have been less mixed, with activation of the D1 receptor just prior to cocaine challenge reported to either decrease or have no effect on sensitization (Sorg et al. 2001; Beyer and Steketee 2002) and activation of the D2 receptor just prior to cocaine challenge reported to decrease sensitization (Beyer and Steketee 2002).

One theory regarding the role of DA input to the mPFC in psychostimulant-induced locomotion suggests that DA can act in either an excitatory or inhibitory fashion, in other words, as a neuromodulator (Steketee 2003). As noted above, preventing cocaine-induced activation of D2 receptors in the mPFC decreases cocaine-induced

hyperactivity (Beyer and Steketee 2000), which is similar to the findings presented here showing reduced AMPH- or METH-induced hyperactivity following D1 receptor blockade in the mPFC. The fact that inactivation of either receptor type in the mPFC can lead to similar effects on psychostimulant-induced behavior is understandable in light of evidence that D1 and D2 receptors are differentially localized in the mPFC, with only minimal co-localization on interneurons (Al-Tikriti et al. 1992; Vincent et al. 1993, Vincent et al. 1995). Activation of either receptor subtype has been reported to lead to decreases in glutamate levels in the mPFC (Abekawa et al. 2000; Harte and O'Connor 2004), which could reflect decreased activity of the pyramidal output cells. This could in turn lead to similar overall effects on psychostimulant-induced hyperactivity. Of course, it is likely that other neurotransmitter systems within the mPFC, particularly norepinephrine (Shoblock et al., 2004), 5-HT (Kuroki et al. 1996), and acetylcholine (Day and Fibiger 1992), also play a role in the behavioral responses studied here. While this may have contributed to the incomplete blockade of AMPH- and METH-induced locomotor activity by SCH 23390, it is clear that DA actions at D1 receptors play a particularly important role.

The effects of SCH 23390 on METH-induced, and most likely on AMPH-induced, hyperactivity was not due to SCH 23390's effects on 5-HT_{2C} receptors: co-infusion of the 5-HT_{2C} antagonist RS 102221 with SCH 23390 did not alter the effects of SCH 23390 alone on METH-induced locomotion. Co-infusion of RS 102221 did tend to further decrease the SCH 23390-mediated block of rearing activity, however. Infusion of RS 102221 alone also tended to reduce METH-induced rearing while having no effect on METH-induced locomotion. These results suggest that METH-induced rearing and

horizontal activity may be mediated in the mPFC by mechanisms that are at least partially distinct, with the former being more significantly influenced by activity at 5-HT_{2C} receptors. In this regard, it is interesting that increased levels of 5-HT are found in the PFC of rats that exhibit high levels of rearing activity in response to stress (Antoniou et al. 2007). Because these issues have not yet been extensively investigated, further studies are necessary to more directly test if there is a distinctive role of mPFC 5-HT_{2C} receptors in METH-induced rearing.

Previously, Ramos et al. (2005) reported that intra-mPFC co-infusion of 0.15 μg/side RS 102221 along with 0.1 μg/side SCH 23390 blocked the effects of SCH 23390 on MDMA-induced hyperactivity and locomotor sensitization. It is likely that the relatively higher potency for releasing 5-HT that MDMA possesses, compared to AMPH and METH (Rothman et al. 2001), contributes to these different results. However, it is interesting to note that 5-HT_{2C} receptors are localized on GABAergic interneurons within layers V and VI (Liu et al 2007), which is similar to the localization of D1 receptors (Vincent et al. 1993). It is also noteworthy that intra-mPFC infusion of 5-HT_{2C} antagonists alters cocaine-induced DA release in the nucleus accumbens (Leggio et al. 2009) and attenuates cocaine-induced hyperactivity (Filip and Cunningham 2003). While these studies point towards a role for 5-HT_{2C} receptors in cocaine-induced activity, our results suggest 5-HT_{2C} receptor activation does not mediate METH-induced locomotion. This hypothesis should be addressed in future studies that use a range of RS 102221 doses.

In summary, the results of this study demonstrate an important contribution of D1 receptor activation in the mPFC to AMPH- and METH-induced locomotor activity.

Given the role of the mPFC as a component of the mesolimbic DA system, these results may have broader implications for other aspects of psychostimulant-induced behavior. For example, AMPH-induced disruption of responding in a 5-choice serial reaction time task can be reversed by intra-mPFC infusion of a D1 receptor agonist (Fletcher et al. 2007). AMPH-induced sensitization can impair attentional set shifting, however this impairment can also be reversed by an intra-mPFC infusion of a D1 agonist (Fletcher et al. 2005). Furthermore, pre-pulse inhibition can be disrupted by either intra-mPFC AMPH or an intra-mPFC D1 antagonist (Swerdlow et al. 2005). Lastly, conditional discrimination created by a tone or click indicating the correct lever to press can be disrupted by systemic AMPH and attenuated by intra-mPFC administration of D1 and D2 receptor antagonists (Dunn and Killcross 2007). These examples, along with the findings from the study presented here, indicate that an understanding of the contribution of D1 receptors within the mPFC is important to an overall understanding of neural mechanisms underlying psychostimulant-induced behavior.

vi. Figures and Figure Legends

Figure 5.1

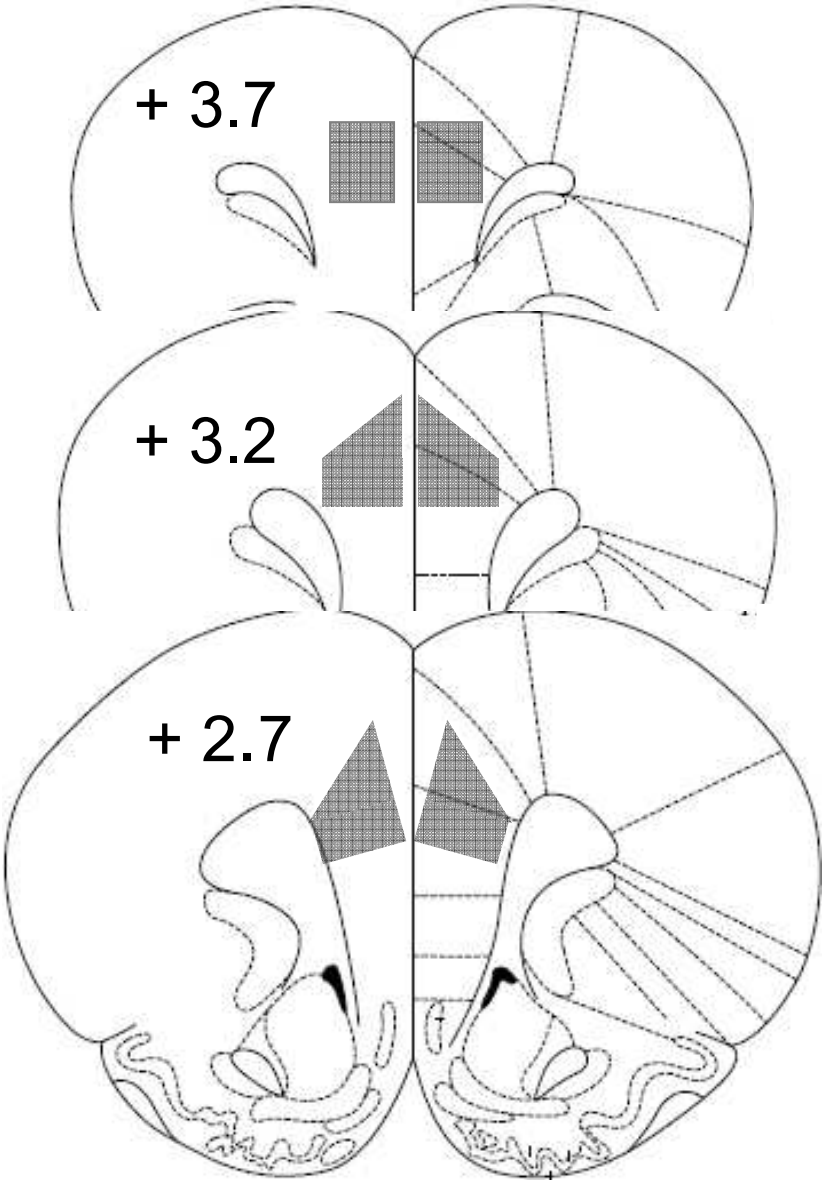

Figure 5.1 Schematic representation of sites for local infusions in the mPFC. Due to the large number of individual cannulae placements, shaded boxes are used in the diagram to indicate the area within which all placements were localized. Numbers (in mm) indicate the coronal section position relative to Bregma (sections adapted from Paxinos and Watson, 1998).

Figure 5.2

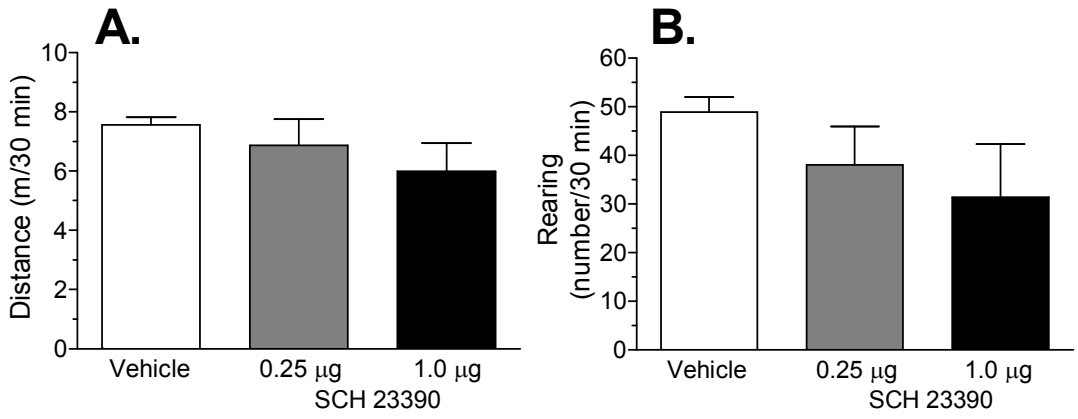

Figure 5.2 Locomotion (A) and rearing (B) in an open-field arena during the 30 min period that followed intra-mPFC infusion of vehicle (n = 48) or the D_1 antagonist SCH 23390 (n = 6-7/group) and systemic injection of saline. Data in this and subsequent figures are presented as mean ± SEM

Figure 5.3

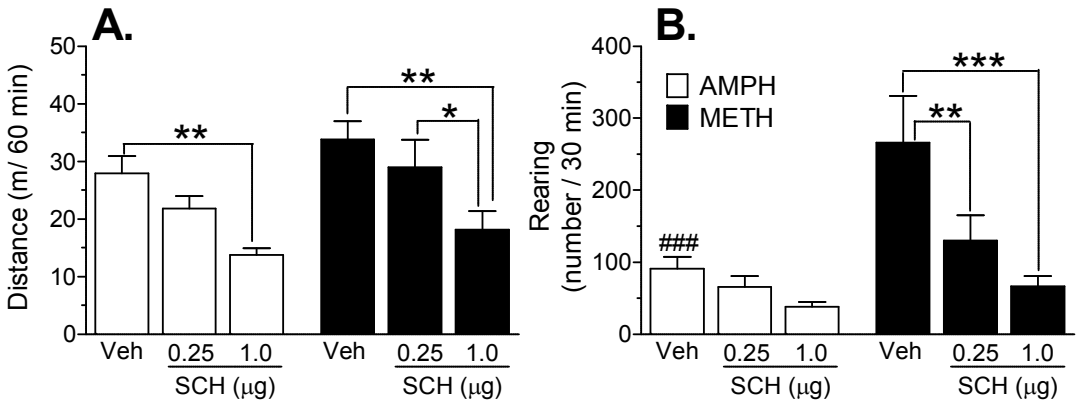

Figure 5.3. The effects of intra-mPFC infusions of vehicle or SCH 23390 on AMPH- or METH-induced locomotion (A) and rearing (B) in an open-field arena. Shown is the cumulative activity for the 60 min period following injection of 1.0 mg/kg (i.p.) AMPH or METH. Rats (n = 7-10/group) were infused with vehicle or SCH 23390 (0.25 or 1.0 μg/side) 15 min before systemic drug injections. Because of data loss due to an equipment malfunction, rearing data for the group given intra-mPFC SCH 23390 (0.25 μg/side) and AMPH are based on n = 6. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$, comparisons between specified groups; $^{\#\#\#}p < 0.001$, compared to rats given METH and intra-mPFC infusions of vehicle.

Figure 5.4

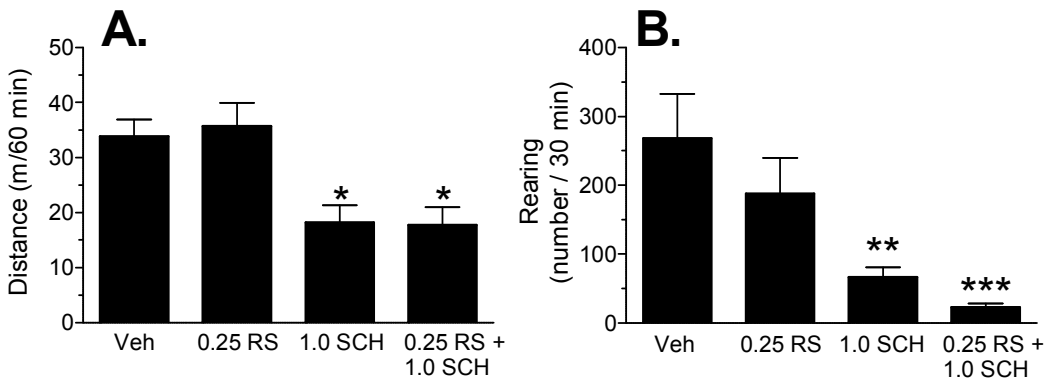

Figure 5.4 The effects of intra-mPFC infusions of the 5-HT_{2C} antagonist RS 102221, alone or in combination with SCH 23390, on METH-induced locomotion (A) and rearing (B). Rats (n = 6-10/group) were given intra-mPFC infusions of vehicle, RS 102221 (0.25 µg/side), SCH 233090 (1.0 µg/side), or RS 102221(0.25 µg/side) in combination with SCH 23390 (1.0 µg/side), 15 min before 1.0 mg/kg (i.p.) METH. The data from the groups given intra-mPFC vehicle or 1.0 µg/side SCH 23390 are the same as shown in Fig. 3. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ compared to rats given intra-mPFC infusions of vehicle.

Chapter 6. General Discussion

One theory in the field of addiction that has gained importance over the years is that many drug-related behaviors, such as relapse, can be traced back to maladaptive associative learning (O'Brien et al. 1990; Field and Cox 2008). The process by which this occurs is thought to be similar to Pavlovian conditioning wherein a previously neutral CS takes on predictive value for an US. Eventually the CS takes on the ability to drive behavior related to obtaining the US (Wikler 1973; O'Brien et al. 1992). The purpose of this thesis was to examine two particular psychostimulants, METH and AMPH, and their effect on associative learning processes. In addition, the roles of two specific neurobiological mechanisms, glucocorticoid and dopamine receptor activation, were examined to determine their possible contribution to these processes.

The experiments presented here evolved primarily from our initial study comparing the ability of relatively low-dose METH and AMPH to induce sensitization, and how additional salient stimuli may affect this response. We found that overall METH and AMPH are equipotent at eliciting behavioral activation, but appear to have differential effects on measures of associative learning. Particularly, the addition of salient stimuli to the testing chamber increased METH-induced behavior to a greater extent than AMPH-induced. Additionally, rats pre-treated with METH exhibited cross-sensitization to AMPH, but the same was not true of rats pre-treated with AMPH then given METH. Upon first inspection the differing effects of cross-sensitization can be easily dismissed due to the fact that AMPH is a metabolite of METH. Therefore, the METH-treated rats have experienced AMPH, but the AMPH treated-rats have never before experienced METH. However, AMPH has been shown to cross-sensitize with

other drugs whose metabolites are not AMPH, like cocaine and nicotine (Liu et al. 2007; Santos et al. 2009). Moreover, not all psychostimulants cross-sensitize with METH, such as methylphenidate, which has similar pharmacodynamics as AMPH (Kuczenski and Segal 2002). It is still not clear why AMPH did not cross-sensitize with METH, but taken together with the enhanced effects of METH in the presence of salient stimuli these results suggest that METH may have a greater impact on associative learning.

We then utilized a different paradigm to further examine the role of METH and AMPH in associative learning, with a particular focus on how these drugs would modify the relationship between stimuli and reward-response. The PIT technique was chosen with the purpose of providing a more direct method to examine the influence of associative learning on behavior. We originally hypothesized that the administration of psychostimulants following Pavlovian approach training would enhance transfer on the PIT test. Instead both drugs unexpectedly interfered with transfer, with METH altering behavior to a greater extent than AMPH. One possible explanation for this result may be the timing of testing in relation to drug administration. By testing only 72 hours following the final administration of METH or AMPH, PIT may have been affected by neurochemical events related to acute withdrawal.

It should be noted that one other group found a similar decrease in PIT when a drug was administered immediately following sessions during the Pavlovian approach phase (Zorawski and Killcross 2003). In this case, a GR agonist, dexamethasone, was administered immediately following Pavlovian approach training. This resulted in increased Pavlovian approach, but a decreased transfer effect. The authors of this manuscript hypothesized that by enhancing CS-US association during Pavlovian

approach, greater importance was placed on the US. This in turn would result in decreasing the importance of the sensory stimuli (i.e. the levers) that signal reward-availability, thus attenuating the transfer effect (Zorawski and Killcross 2003). However, the authors did not mention any examination of measures that would indicate an increase in US-directed behavior, such as an increase in number of trough approaches or time spent in the trough. Even though the differences we observed in Pavlovian approach were non-significant, there was a tendency for enhancement of Pavlovian approach in drug-treated groups, particularly in the highest doses. To examine the possibility of increased trough-directed behavior interfering with instrumental behavior, we performed a close examination of behavior on the PIT testing day. However, no evidence was found to support excessive trough-directed activity. It should be noted that there are other measures of trough-directed activity that our equipment is not set up to measure, such as location of the rat within the box and number of approaches toward the trough.

One final possible explanation for our results lies with the possibility that the PIT technique is not a direct measure of the salience of the Pavlovian approach CS. If the hypothesis put forth by Zorawski and Killcross is correct, then the concept of PIT as a measure of the motivational effect of a CS on instrumental behavior is called into question. In accordance with the original premise of PIT, enhanced association between a CS and US would lead to increased, rather than decreased, instrumental behavior due to the enhanced motivational or salient value of the CS. Thus, a decrease in instrumental behavior should indicate decreased CS salience (Estes 1948; Rescorla and Solomon 1967; Everitt et al. 2001). The results of Zorawski and Killcross, as well as those found

here, in fact indicate the opposite effect is true; that enhancing CS salience decreases rather than increases instrumental behavior.

Further evidence for the possibility that PIT may not be direct measure of the salient value of a CS rests in the fact that the transfer effect is most evident when a longer, i.e. 30 sec or greater, CS is utilized (Crombag et al. 2008; Delameter and Holland 2008). Conversely, a much shorter CS, i.e. 10 sec or less, is most commonly utilized for experiments solely examining the effect of manipulations on Pavlovian approach. Longer CSs in a Pavlovian approach paradigm tend to lead to decreased acquisition of specific goal-directed behavior. Thus, the transfer effect is most apparent when Pavlovian conditioned approach is only weakly evident. Therefore, it would seem that PIT is actually an expression of a balance between the motivational value of the CS and the salience of the instrumental stimuli (i.e. levers); wherein if the CS gains an inordinate amount of salience the transfer effect is lost.

Even though the results from Experiment 2 were the inverse of those originally hypothesized, METH was found to have a greater impact on associative learning than AMPH. This result supports those of Experiment 1 indicating that METH-administration may have a greater capacity to influence associative learning processes than AMPH. To understand how METH and AMPH acted to affect the changes observed in Experiment 1, two potential neurobiological mechanisms were examined. First described here was the contribution of glucocorticoid receptors to METH- and AMPH-induced sensitization. Glucocorticoids have been shown to significantly impact responding to psychostimulants, but a direct examination of whether activation of GRs contributes to induction or expression of sensitization has not been undertaken until now. Our results indicate that

METH- and AMPH-induced locomotor sensitization is differentially affected by GR inactivation. Overall, locomotor activity induced by METH was resistant to alterations produced by GR inactivation, while AMPH-induced activity was decreased when a GR antagonist was administered on the challenge day. Conversely, inactivation of GRs had a greater anxiolytic effect on METH-sensitized rats. This suggests that the development of METH-induced locomotor sensitization occurs independently of GR activation. Taken together, these results indicate that GRs play a differential role in METH- and AMPH-induced locomotor sensitization.

The second possible mechanism examined was the role of mPFC DA D1 receptors in the acute drug response. In this study we focused on the initial response to these drugs because it has been demonstrated that associations formed during initial exposure are particularly effective at influencing later drug-related behaviors. For instance, re-exposure to contextual cues that were present at a single cocaine exposure can increase cocaine-seeking up to one year later (Ciccocioppa et al. 2004). Both METH- and AMPH- induced locomotor activity was examined due to previous reports that METH may enhance DA transmission within the mPFC to a greater degree than AMPH (Shoblock et al. 2003). Thus, we hypothesized that AMPH-induced activity may be more vulnerable to D1 receptor manipulation due to less DA available for D1 receptor binding. The D1 receptor blockade dose-dependently decreased psychostimulant-induced locomotor activity; however we found no difference in AMPH- and METH-induced activity. Since a blockade of mPFC D1 receptors does not affect spontaneous locomotor activity, it can be inferred that activation of mPFC D1 receptors is critical for

psychostimulant-induced locomotor activity but do not differentially influence METH- and AMPH-induced locomotion.

To summarize, in the experiments presented here both METH and AMPH were found capable of altering associative learning, but METH appears to have a greater impact on these processes than AMPH. It is possible that these differential effects are mediated by activation of GRs. The initial locomotor response to both of these drugs is heavily dependent upon D1 receptor activation in the mPFC, but this is most likely not the mediating factor in the differences between the two drugs. These findings indicate that the differing abuse liabilities and suggestions of varying potencies between METH and AMPH may be partially predicated by their influence on associative learning processes. Furthermore, overlapping, but somewhat distinct neurobiological mechanisms likely govern these drug-induced differences in associative learning.

Chapter 7. Works Cited

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